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Biological Sciences

EFFECTS OF PRESCRIBED BURNING ON HERBACEOUS VEGETATION AND POCKET GOPHERS (*Geomys pinetis*) IN A SANDHILL COMMUNITY

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ABSTRACT: Effects of season of prescribed burning and time elapsed since last burn on herbaceous biomass and species composition, root carbohydrate and nitrogen content, and southeastern pocket gopher (*Geomys pinetis*) mounding activity and body condition were examined on a sandhill community. Treatments included late-winter and late-summer burns in 1983, 1984, and 1985, and an unburned control. Post-burn data collected in the fall of 1985 showed no significant differences ($P > 0.05$) for total biomass of aboveground herbaceous vegetation. Aboveground biomass of grasses was higher than forb biomass for all treatments. Forb biomass was lowest on the unburned control. Shannon diversity indices were consistently lower on unburned than on burned treatments. Carbohydrate levels in roots of selected herbaceous species collected from each treatment in the winter of 1986 were variable and no significant differences ($P > 0.05$) were detected. Nitrogen levels in roots tended to be higher on unburned than on burned treatments. Mounding activity varied widely both among and within treatments and ranged from 0.73 to 5.19 mounds produced per day during the winter of 1986. No significant correlation between mounding activity and carbohydrate or nitrogen levels of selected plants was detected. No significant differences ($P > 0.05$) were detected in weight: body length ratios of pocket gophers among treatments during late winter-early spring.

FIRE plays a major role in maintaining Florida sandhill communities of longleaf pine (*Pinus palustris*) and turkey oak (*Quercus laevis*). Increased fire protection over the years has resulted in succession of these xeric, park-like savannas toward hardwood hammock or xeric hardwood-mixed pine communities, with a decrease in herbaceous species diversity (Laessle, 1942, 1958; Vogl, 1972; Myers, 1985).

As a result of removal of accumulated litter, biomass yields of herbaceous species often are increased following fire (Weaver and Rowland, 1952; Grelen and Epps, 1967; Hulbert, 1969). Litter removal also alters the microclimate of emerging vegetation through warmer soil temperatures and increased light which leads to rapid growth of grasses (Old, 1969; Peet et al., 1975).

Changes in species composition frequently accompany burning. Arata (1959) reported that composites and partridge pea (*Cassia fasciculata*) were

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more common on burned than unburned sites in a longleaf pine-turkey oak association in north Florida. In other Florida sandhill communities, forbs and legumes had greater biomass and species richness on one-year-old burns than on two-, three-, or four-year-old burns (Harlow and Bielling, 1962).

Nutritional status of plants also is affected by fire. Chapin (1981) noted that new growth of species that resprout immediately following fire is supported by stored carbohydrate reserves and any current photosynthate. On a wet flatwoods site in south Florida, higher total nonstructural carbohydrate (TNC) levels were found in creeping bluestem (*Schizachyrium stoloniferum*) plants on burned range than on unburned range during the growing season following a February burn (Kalmbacher, 1983). Nitrogen percentages in roots and crowns of grasses on unburned areas of Florida range decreased little throughout the growing season while similar parts of plants from areas burned the previous spring showed significant decreases in percent N during the middle of the growing season (Leukel and Stokes, 1940).

A native herbivore in Florida's sandhill communities is the southeastern pocket gopher (*Geomys pinetis*). Although food habitats of *G. pinetis* are relatively unknown, Ross (1976) reported finding bahiagrass (*Paspalum notatum*), dog fennel (*Eupatorium* spp), lambsquarters (*Chenopodium* spp), and false moneywort (*Alysicarpus vaginalis*) in food caches of this species. Barrington (1940) found that captive animals consumed nutgrass (*Cyperus esculentus*), bermudagrass (*Cynodon dactylon*) and wiregrasses (*Aristida* spp and *Sporobolus* spp). Burrowing activity, indicated by the presence of soil mounds, varies seasonally due to changes in feeding habits, breeding activity, and soil moisture (Miller and Bond, 1960; McNab, 1966; Ewel, 1970; Hickman and Brown, 1973). Mounds produced by *Geomys attwateri* were larger on burned areas where dicots (preferred food plants) had decreased, as compared to unburned areas (Spencer et al., 1985). They suggested that this indicated increased foraging activity. Tilman (1983) estimated responses of *G. bursarius* to fertilizer treatments on range grasses by conducting counts of newly-produced mounds and found positive relationships between total plant biomass and mounding activity.

This study was designed to further investigate the premise that prescribed fire in sandhill communities will alter species composition and nutritive value of herbaceous plants and that these vegetative responses will affect body condition and mounding activity of the southeastern pocket gopher.

DESCRIPTION OF STUDY AREA—The study was conducted on a sandhill community site in the Katherine Ordway Preserve-Swisher Memorial Sanctuary, Putnam County, Florida. Longleaf pine-turkey oak savannas make up about one-third of the 3,640 ha Preserve (Humphrey et al., 1985). Major components of the herbaceous vegetation include pineland threeawn (*Aristida stricta*) and pineywoods dropseed (*Sporobolus junceus*), as well as a number of bluestem grasses (*Andropogon* spp and *Schizachyrium* spp) and a variety of forbs. Soil types include Candler fine sand, Apopka sand, and Astatula fine sand (Putnam County Soil Survey, unpublished).

Logging of pines and frequent winter fires that killed turpentine trees prior to 1970 allowed turkey oak to increase considerably. Prior to 1974, sandhill areas in the western portion of the Preserve were burned periodically in the winter at 3 or 4 year intervals, while sandhill areas in

the eastern portion were burned annually (T. Perry, 1985). The Preserve was leased for cattle grazing prior to 1979. In 1980, the University of Florida acquired the property; subsequently, cattle grazing was excluded and fire frequency altered (Humphrey et al., 1985).

METHODS—Vegetation parameters and pocket gopher activity were measured on six prescribed burn treatments; late-winter burns in March or early April and late-summer burns in late July, August, or early September of 1983, 1984, and 1985. Two unburned areas served as controls. One control area had been burned every 3 to 4 years and the other burned annually prior to 1974. There were two replications of burns in winter 1984, winter 1985, and summer 1985. The 11 study sites ranged in size from 25 ha to 170 ha. Sites burned during winters of 1983, 1984, or 1985 are designated as W83, W84, or W85, respectively. Treatment sites burned during the summers of 1983, 1984, or 1985 are designated S83, S84, or S85, respectively. Unburned control sites are designated CONTROL.

Pre- and post-burn herbaceous species composition and aboveground biomass were estimated using the quadrat-clip technique (National Academy of Sciences-National Research Council, 1986). Pre-burn measurements were taken 1 to 2 months prior to burning on W85 (W85A and W85B) and S85 (S85A and S85B) sites. Ninety-nine 0.5-m² quadrats were placed at 25 m intervals along three randomly located transects of 800 m length on sites W85A, S85A, and S85B; 33 0.5-m² quadrats were placed along one transect of 800 m length on site W85B. Post-burn herbaceous species composition and aboveground biomass were estimated during October through December 1986. Sixty 0.5-m² quadrats were placed at 25 m intervals along randomly located transects within each study site. Vegetation was hand-clipped at ground level, separated by species and weighed in the field. A portion of the samples from each site was oven-dried at 70°C for 72 hours and re-weighed to establish dry weight conversions. The number of samples collected for dry-weight conversions was dependent on variability of weather conditions during the sampling period. The Shannon index was used as the measure of diversity (Shannon and Weaver, 1949).

Mound counts were made to determine relative mounding activity levels of pocket gophers (Tilman, 1983). Three, square 1.0-ha quadrats were established on each of the 11 study sites. The size of quadrat selected was based on Brown and Hickman's (1973) estimate of average tunnel system length in south Florida. In September 1985, new mounds showing little or no evidence of weathering were selected as center points for each quadrat. Continuing through September and part of October, all existing pocket gopher mounds within the quadrats were flagged and the date recorded. Counts of new mounds were made in December and January, months gophers previously had been reported to be most active, to serve as an index of activity (Hickman and Brown, 1973). An index of mounding activity in each quadrat was calculated by dividing the number of new mounds counted in 32 to 39 day intervals during December and January by the number of days since the previous count.

From late January to early March 1986, belowground parts of six plant species, pineland threeawn, pineywoods dropseed, creeping bluestem, grassleaf goldaster (*Heterotheca graminiifolia*), false deertongue (*Carphephorus corymbosus*), and wild buckwheat (*Eriogonum tomentosum*), were collected in the pocket gopher mound activity quadrats for carbohydrate and nitrogen analyses. Belowground plant parts, hereinafter referred to as roots, included roots, rhizomes, and belowground crown portions of some species. Roots of at least five plants of each species were collected and combined to give a composite sample of each species for each quadrat. Extraction procedures for determining carbohydrate content of the root samples followed those of Rowe (1980) with minor modifications (Gates, 1986). Microkjeldahl digestion of root tissues followed Nelson and Sommers (1973) and ammonium in the digest was determined using procedures outlined by Bremner and Edwards (1965).

A minimum of three pocket gophers was captured in each of the 11 study sites between 2 February and 9 April 1986 using a modified version of the Sherman pocket gopher trap (Sherman, 1941). Captured animals were weighed, sexed, and body length measurements taken prior to release back into the tunnel system. Body weight-to-body length ratios were used as an index of condition.

Data were analyzed using a general linear model (GLM) for least squares regression (Statistical Analysis Institute, 1982). When GLM procedures detected significant differences ($P < 0.05$), *t*-tests of least significant differences (LSD) were carried out to determine differences between specific treatments. Contrast analyses (Statistical Analysis Institute, 1982) were used to detect season and year effects and season by year interactions. Vegetation data were log-transformed for analysis, but all tabular data appear as actual mean values.

RESULTS AND DISCUSSION—Herbaceous Vegetation—Fifty-two herbaceous species were encountered during the study (Gates, 1986). Pineland threeawn, pineywoods dropseed, and purple threeawn (*Aristida purpurascens*), were the most common grasses and grassleaf goldaster was the most common broadleaved species. Overall, pineland threeawn had the highest frequency of occurrence (73.3%) and the greatest mean biomass (35.2 g/m²) of all species. Frequency of occurrence of forbs (all species combined) was considerably lower in the CONTROL than among the burn treatments (Table 1). However, no trend in forb frequency was apparent among the burn treatments. Overall, frequency of occurrence of grasses was much higher than for forbs; however, differences in grass frequency between the burn treatments and the CONTROL were minimal. In northwest Florida, Vogl (1972) found higher forb cover on plots burned at 1-, 2-, or 3-year intervals than on unburned plots but differences were less dramatic than for grass cover. Aboveground biomass of grasses was considerably higher than forb biomass among burn treatments (Table 1). Although biomass generally was lower in the CONTROL for both plant groups, increases in forb biomass were proportionately much higher in burn treatments as compared to overall grass responses. The lower S85 yields were the result of late-season burns that consumed most of that year's growth.

TABLE 1. Frequency of occurrence (%) and aboveground biomass (g/m²) of forbs and grasses on sandhill community treatments, Ordway-Swisher Preserve, Putnam County, Florida.

Treatment	Forbs		Grass	
	Frequency	Biomass	Frequency	Biomass
CONTROL	25.0	2.0	93.3	30.0
W83	51.7	8.0	96.7	53.9
S83	38.3	7.6	95.0	46.2
W84	50.0	14.2	95.0	65.4
S84	50.0	7.3	90.0	36.1
W85	65.0	32.0	91.7	76.9
S85	50.0	4.2	90.8	17.0
Winter Average	56.3	20.1	94.0	67.7
Summer Average	47.1	5.8	91.7	29.1

Winter-burned treatments had greater forb and grass biomass than summer-burned treatments, in each respective year. However, GLM and contrast procedures detected no significant differences among treatments for forb or grass biomass. Lewis et al. (1965) reported that grass production in Ozark forests was higher when burned in March or April than in June or August but that forb production was greatest following August burning.

Forbs generally respond rapidly to fire. Annual species have reduced competition from perennials for sunlight, at least temporarily, and litter removal provides a more favorable seedbed (Hodgkins, 1958; Vogl, 1972). Plants with wind-dispersed seed, such as composites, quickly invade and increase in abundance on newly-burned areas (Lemon, 1949; Arata, 1959), while some forbs, including a number of legumes, benefit from seed scarification effects of fire (Stoddard, 1931; Garren, 1943). In this study, few annual

species were encountered. This may be due to the long-term exclusion (up to 12 years) of fire which resulted in fewer seed sources for annual species.

Pineland threeawn was the dominant herbaceous species present, comprising more than 50% of the total herbaceous biomass in all treatments. Statistical comparisons of pineland threeawn biomass among the treatments, however, showed no significant differences (Table 2). Greatest biomass of this species was in treatments W85 and W84 with 52.8 and 51.4 g/m², respectively. Harlow and Bielling (1962) reported that wiregrasses (*Aristida* spp) reached a peak in abundance 3 years after burning. It is of interest that pineland threeawn had greater biomass at 8 and 20 months post-burn in this study. Although the means of each treatment suggest a significant season effect, high variation obscured any significance in differences.

TABLE 2. Means and standard deviations of aboveground biomass (g/m²) of pineland threeawn on sandhill community treatments, Ordway-Swisher Preserve, Putnam County, Florida.

Treatment	Biomass	Months Since Burn
CONTROL	25.7 ± 35.7	140
W83	42.6 ± 63.0	32
S83	34.9 ± 40.5	26
W84	51.4 ± 69.4	20
S84	27.1 ± 36.7	14
W85	52.8 ± 59.0	8
S85	12.5 ± 18.0	2
Winter Average	50.2	
Summer Average	21.8	

Pre-burn herbaceous biomass data showed that the W85 burn sites (W85A and W85B) were not significantly different (Table 3). Thus differences between these sites in post-burn vegetation biomass may be primarily attributable to the specific burn event. S85 sites were significantly different from each other prior to burning with S85B having greater mean herbaceous biomass pre-burn and post-burn. Interestingly, burning reduced herbaceous biomass on both S85 sites by ca 50 %.

TABLE 3. Comparison of pre-burn and post-burn means of aboveground herbaceous biomass (g/m²) for W85 and S85 study sites, Ordway-Swisher Preserve, Putnam County, Florida.

Site	Herbaceous vegetation	
	Pre-burn	Post-burn
W85A	38.9ab	143.4*
W85B	33.1bc	76.8*
S85A	31.5c	16.5*
S85B	52.3a	26.6*

*Indicates post-burn mean is significantly different ($P < 0.05$) from its respective pre-burn mean; means with the same subscript letter are not significantly different.

Post-burn diversity indices using both biomass and frequency data from winter and summer treatments of 1985 were larger than pre-burn indices (Table 4). This indicates that diversity tends to increase shortly after burning. Post-burn diversity indices based on biomass and frequency data for all treat-

ments and all years indicate that diversity was greater on burned areas compared to the unburned CONTROL (Table 5).

TABLE 4. Comparison of pre-burn and post-burn Shannon diversity indices for biomass and frequency of occurrence of herbaceous plants in 1985 on a sandhill community, Ordway-Swisher Preserve, Putnam County, Florida.

Treatment	Biomass		Frequency	
	Pre-burn	Post-burn	Pre-burn	Post-burn
W85	1.31	2.08	2.15	2.98
S85	1.62	1.89	2.26	2.72

Other authors also have reported increased diversity due to fire (Vogl, 1972, Shafi and Yarranton, 1973; Moore et al., 1982). Diversity increased in turkey oak sandhills protected from fire for 40 years (Veno, 1976). The greatest change in diversity levels was attributed to an increase in mesic hammock species. Veno concluded that with long-term protection from fire, a number of fire-intolerant species could invade these sites.

TABLE 5. Post-burn Shannon diversity indices for biomass and frequency of herbaceous plants on sandhill community treatments, Ordway-Swisher Preserve, Putnam County, Florida.

Treatment	Biomass	Frequency
CONTROL	1.04	2.55
W83	1.46	2.99
S83	1.71	2.91
W84	1.72	2.86
S84	1.82	2.93
W85	2.08	2.98
S85	1.89	2.72

TABLE 6. Total nonstructural carbohydrate (TNC) levels (mg/g) in roots of selected herbaceous species, Ordway-Swisher Preserve, Putnam County, Florida.

Species	Control	Year of Burn			Season of Burn	
		1983	1984	1985	Winter	Summer
Pineland threeawn	104	75 ^a *	73 ^a *	78 ^a *	76 ^a *	75 ^a *
Pinewoods dropseed	26	30 ^a	30 ^a	25 ^b	30 ^a	25 ^a
Creeping bluestem	144	144 ^a	148 ^a	134 ^a	151 ^a	128 ^b
Grassleaf goldaster	147	152 ^a	131 ^a	160 ^a	145 ^a	153 ^a
False deertongue	142	153 ^a	148 ^a	176 ^a	159 ^a	163 ^a
Wild buckwheat	216	212 ^a	215 ^a	221 ^a	214 ^a	221 ^a

*Indicates yearly or seasonal mean significantly different from control; for a given species, means with the same subscript letter are not significantly different.

Total Nonstructural Carbohydrates—Total nonstructural carbohydrate (TNC) concentrations in most species generally were not affected by burning when compared to the CONTROL (Table 6). TNC generally was higher in forbs than in grasses except for creeping bluestem, which was similar to grassleaf goldaster and false deertongue in winter-burned treatments.

TNC values for pineland threeawn were significantly lower for all burn treatments than for the CONTROL. Creeping bluestem was the only species

that had significantly different TNC levels due to summer vs. winter burning. TNC was higher with winter burning, which was a general trend for all grasses. TNC in forbs tended to be somewhat higher with summer burns. It is interesting to note that TNC in pineywoods dropseed was 4- to 7-times lower than any other species.

No clear pattern in TNC concentrations as affected by burning is discernible. However, defoliation through burning may have differential effects within the relatively short time periods since burning. Trlica and co-workers (1977) reported differential recovery rates following defoliation among western range species. Some species, such as fringed sagewort (*Artemisia frigida*) and rabbitbrush (*Chrysothamnus vicidiflorus*), showed lower TNC levels in roots of defoliated plants even after 14 to 26 months of rest. This wide variation may be due to differences in root morphology and physiology, and/or to microsite or macrosite conditions at the time of sampling.

TABLE 7. Nitrogen levels (%) in roots of selected herbaceous species, Ordway-Swisher Preserve, Putnam County, Florida.

Species	Control	Year of Burn			Season of Burn	
		1983	1984	1985	Winter	Summer
Pineland threeawn	0.45	0.30 ^a *	0.31 ^a *	0.34 ^a *	0.33 ^a *	0.31 ^a *
Pineywoods dropseed	0.31	0.28 ^a	0.28 ^a	0.32 ^a	0.30 ^a	0.30 ^a
Creeping bluestem	0.49	0.42 ^a	0.37 ^a	0.42 ^a	0.45 ^a	0.34 ^a
Grassleaf goldaster	1.15	0.76 ^a	0.70 ^a	0.83 ^a	0.69 ^a	0.87 ^a
False deertongue	0.99	0.64 ^a	0.58 ^a	0.59 ^a	0.48 ^a *	0.72 ^b
Wild buckwheat	0.59	0.36 ^a	0.55 ^{ab}	0.62 ^b	0.46 ^a	0.64 ^a

*Indicates yearly or seasonal mean significantly different from control; for a given species, means with the same subscript letter are not significantly different.

Nitrogen Concentrations—Average nitrogen (N) levels were somewhat lower for all species after burning (Table 7). Differences generally were not significant, except that yearly means for pineland threeawn were significantly lower than the CONTROL. For false deertongue, winter-burned treatment means for the 3 years combined were significantly less than the CONTROL also. Percent N in false deertongue was significantly higher with summer burns than with winter. Pineland threeawn and pineywoods dropseed had very similar N concentrations. Overall, grasses tended to have lower N levels than forbs.

Woods and co-workers (1959) studied seasonal variation in nitrogen levels of pineland threeawn roots in sandhill communities in western Florida where levels were about 0.48 % during the months of January, February and March. This was comparable to the 0.45 % N levels for pineland threeawn in our CONTROL under similar conditions. Old (1969) reported that N content of roots from burned plots in an Illinois prairie grassland were significantly lower than in unburned plots and attributed this to root N depletion by rapidly growing aboveground vegetation. Leukel and Stokes (1940) found that N percentage of roots of Florida range grasses decreased during the middle of the growing season in areas burned the previous spring, with percentages increasing in the fall.

TABLE 8. Mean number of mounds produced per day during December and January, 1986, by pocket gophers on sandhill community treatments, Ordway-Swisher Preserve, Putnam County, Florida.

Treatment	Mounds/day		
	DEC	JAN	Mean
CONTROL	1.38	1.55	1.5
W83	1.51	2.17	1.8
S83	1.49	5.19	3.3
W84	2.46	3.28	2.9
S84	1.43	3.42	2.4
W85	0.88	0.73	0.8
S85	1.35	2.21	1.8
Winter Average	1.64	2.04	1.8
Summer Average	1.40	3.26	2.3

Pocket Gopher Mounding Activity—Mounding activity was quite variable among and within the study sites and no significant differences were detected among treatments, years or seasons (Table 8). Mounding activity usually was greater on burned treatments except for the 1985 winter burn. Hickman and Brown (1973) noted seasonal variation in mounding activity of *G. pinetis* with a peak in December. They also reported a high degree of individual variation in number of mounds produced and stated that "patterns of mound production were entirely irregular, varying from one portion of the same system to the other." Thus the differences in mounding activity observed within our quadrats and treatments may have been due to variation among individual animals.

To determine if a relationship existed between plant nutritional levels and pocket gopher mounding activity, scatter diagrams of carbohydrate values (soluble sugars, starch and TNC) and of N values were plotted against number of mounds per day per quadrat. No relationship between mounding activity and nutrient levels was apparent.

Pocket Gopher Body Condition—Three to five individuals were trapped on each study site between 2 February and 9 April of 1986. Of the 45 animals trapped, 30 were females and 15 were males. Weights ranged from 90 to 265 g and body length ranged from 14.0 cm to 20.3 cm. Average weight and body length of male pocket gophers was 207 g and 18.0 cm, respectively. Females had an average weight of 139 g and average body length of 16.8 cm.

To compensate for differences due to sex and age, a ratio of weight to body length for each animal was calculated and used for comparison among treatments (Table 9). There were no significant differences in mean weight:body length ratios among treatments, years, or seasons, although ratios from winter burns were slightly higher than from summer.

Lack of significant differences in growth indices of pocket gophers may be due to high variability in weight:body length ratios and small sample sizes per treatment. However, it is also possible that despite treatments these animals are able to select home ranges within each site that allow them to meet their nutritional needs. Determination of actual numbers of pocket gophers

TABLE 9. Mean weight:body length ratios (g/cm) and number of pocket gophers captured on sandhill community treatments, Ordway-Swisher Preserve, Putnam County, Florida, between 2 February and 9 April 1986.

Treatment	Mean ratio	Number captured*
CONTROL	8.7	2/5
W83	11.0	2/2
S83	9.1	0/3
W84	9.3	4/5
S84	8.8	2/3
W85	10.3	3/5
S85	8.4	2/7
Winter Average	9.9	
Summer Average	8.6	

*M/F = Number of males and number of females.

on each site may more precisely indicate quality of the habitat for *G. pinetis* under different burn regimes.

CONCLUSIONS—Differences among burn treatments were difficult to ascertain due to the great variation in the parameters measured. Wiregrasses, particularly pineland threeawn, continued to dominate the herbaceous layer following fire with no major shifts in frequency of occurrence or biomass production. Winter-burned treatments tended to yield greater herbaceous biomass and higher frequencies of occurrence of forbs and grasses. Each measure of response to burning generally exceeded the level on plots with long-term protection from burning. It is possible that some actively growing plant species were adversely affected by burning during the growing season just prior to seed-set, or that summer burns destroyed the current year's seed crop. All burn treatments had greater diversity than the unburned control. Furthermore, forbs were more diverse on recently burned areas as compared to unburned sites or sites burned 2 years earlier. Impacts of burning on root carbohydrate and nitrogen levels were minor. While periodic prescribed burning has beneficial effects on some sandhill plant species, resulting in increased biomass, no apparent effects, either positive or negative, of burning on pocket gophers were observed at the successional stages examined. From information gathered in this study, it appears that pocket gophers may have a fairly wide range of tolerance to present vegetative conditions in sandhill communities. However, if succession in sandhill communities continued unimpeded toward a hardwood hammock community, leading to a gradual loss of sandhill plant species, pocket gopher populations would eventually decline.

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PURIFICATION AND ACTIVITY OF *PTYCHODISCUS BREVIS* (RED TIDE) TOXIN ASSOCIATED WITH RESPIRATORY PROBLEMS

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ABSTRACT: *This study was initiated to investigate the compound(s) of Ptychodiscus brevis toxin (PBTX) that causes contraction of canine tracheal smooth muscle. The components of PBTX were separated by reverse-phase high performance liquid chromatography (HPLC) using a chloroform-methanol linear solvent gradient system. Purified PBTX fractions were tested for neurological activity (as measured by canine tracheal smooth muscle contraction) and hemolytic activity (as measured by kinetics of hemolysis of canine blood cells in a blood-buffer solution). Chemical analysis was conducted to determine the structure of the neurologically active compound of PBTX.*

The neurological activity of crude PBTX was enhanced by HPLC purification. A 6-fold increase in neurological activity was observed after the first HPLC purification, while a 75-fold increase in neurological activity was demonstrated after the second HPLC purification as compared to crude PBTX. Three primary peaks were observed in the HPLC chromatogram: one (PBTX-2a) had neurological activity, a second (PBTX-1) had hemolytic activity, and a third (PBTX-2b) did not have neurologic or hemolytic activity. In addition, tachyphylaxis (rapidly developing tolerance) occurred in canine tracheal smooth muscle repetitively exposed to PBTX-2a, which was found to have a molecular weight of about 900 daltons and an ultraviolet absorption maximum of 208 nm. These chemical characteristics were observed for an eleven member transused ether ring compound that was neurologically active and whose structure was elucidated by previous researchers.

OVERBLOOMS of planktonic organisms (red tides) can occur when optimal growth conditions exist in the marine environment. Red-tide blooms in the Gulf of Mexico have been associated with large fish kills for at least 100 years (Walker, 1884). In 1948, Davis identified *Gymnodinium brevis* as the causative organism in red tide outbreaks occurring on the Gulf coast of Florida. Steidinger (1979) later noticed an indentation on the thecal ridge of the organism that resulted in its re-classification to *Ptychodiscus brevis*. Large algal blooms, sudden appearance of large numbers of *Ptychodiscus brevis*, impart a reddish tint to the seawater, thus the name red tide. The red color seen during blooms of *Ptychodiscus brevis* could be caused by a bacterium (Evans et al., 1973).

Ptychodiscus brevis toxin (PBTX) poses serious health problems during a red tide outbreak when PBTX becomes aerosolized in the sea-spray (Steidinger et al., 1973; Woodcock, 1948). The symptoms include nonproductive cough, sneezing, and rhinorrhea, which may lead to difficulty in breathing. Individuals who are asthmatics are particularly sensitive to PBTX as

shown in a clinical survey where 12 out of 15 asthmatic patients exposed to PBTX had an asthmatic attack (Asai et al., 1982).

Due to the health dangers associated with PBTX, it is of interest to know the actual compound responsible for the asthma-like symptoms, particularly bronchoconstriction.

Research has been undertaken to determine the physiological response to crude and purified *Ptychodiscus brevis* toxin in various *in-vitro* and *in-vivo* systems. These systems include the following: heart preparations (Johnson et al., 1985; Sasner et al., 1972), isolated cells (Catterall et al., 1984), and isolated nerves (Baden et al., 1984; Shinnick-Gallagher, 1980; Parmentier et al., 1978). Few studies have been undertaken to determine which component of PBTX has activity on smooth muscle of the respiratory tract. Knowing the structure of the neurologically-active PBTX fraction would result in a clearer understanding of the neurological mechanism of PBTX causing canine tracheal smooth muscle contraction, identification or prediction of compounds with neurological activity, a clearer understanding of the asthmatic abnormality, and characterization of a new pharmacological agent that could be used as a research tool.

MATERIALS AND METHODS—Culturing *Ptychodiscus brevis*—The dinoflagellate was grown using batch culture techniques (Brydon et al., 1971; Asai et al., 1982).

Extraction of Crude *Ptychodiscus brevis* Toxin From Cultures—The procedure of Asai et al., 1982 was used. After 3 to 7 weeks of cell growth, cultures were extracted with 1 ml of 12 M hydrochloric acid per liter of culture and 50 ml of pure chloroform per liter of culture (Martin et al., 1970). The resulting crude toxin was then stored in the dark at -17°C.

Purification of *Ptychodiscus brevis* Toxin—Crude PBTX was dissolved in chloroform to give a concentration of 10 mg/ml. The solution was then passed through a 3 cc C-18 Bond Elut® column (Analyti-chem International, Harbor City, CA). Samples ranging from 5 to 100 μ l were injected into a solvent programmable HPLC (Model 110A, Altex) equipped with 2 pumps. A LKB Bromma UV detector measuring absorbance at 254 nm (Model 2238 Uvicord S2) was used and samples were separated through a reverse-phase column (Altex Ultrasphere, octadecylsilane, 5 μ , 4.6 mm inner diameter). The column pressure was approximately 1000 psi at 1 ml/min flow rate. The HPLC was programmed to run a linear gradient for 10 minutes starting at 0% methanol and ending at 30% methanol in chloroform. PBTX fractions were collected manually and stored in the dark at -17°C. The solvent phase was then evaporated under reduced pressure using a rotary evaporator and stored in the dark at -17°C.

Testing Neurological Activity of PBTX on Canine Tracheal Smooth Muscle Strips—Neurological activity was measured using methods previously described (Anderson et al., 1979). Mixed-breed dogs weighing from 15 to 22 kg were anesthetized with intravenous injections of sodium pentobarbital (30 mg/kg). The trachea was then surgically removed and immediately placed in aerated (95% oxygen, 5% carbon dioxide) Krebs-Ringer solution of the following composition (mM): NaCl, 117.0; KCl, 4.0; NaHCO₃, 25; MgSO₄, 2.4; NaH₂PO₄, 1.2; CaCl₂, 2.5; and dextrose, 11.0. Before obtaining smooth muscle strips, mucous membrane and extraneous tissue was removed from the trachea. Smooth muscle strips (2 to 3 by 15 to 20 mm, weighing approximately 30 mg) were dissected from the cartilage. Each strip was tied with surgical thread and mounted in an isolated water jacketed bath containing 10 ml of Krebs-Ringer solution, aerated (95% oxygen, 5% carbon dioxide) and maintained at 37°C, pH 7.4.

Isometric tension was measured with a Grass FT03 force-displacement transducer (Grass Instrument Co., Quincy, Mass.) coupled to a Grass Model 7 polygraph. Each tissue was initially loaded with 2 grams of tension, and 30 minutes was allowed before testing to allow the tissue to equilibrate. This initial tension was tested to assure that responses were measured at the L_{max} for this tissue.

Cumulative acetylcholine concentration-response curves (10^{-8} to 10^{-3} M) were obtained before and after exposure of the tissue to PBTX to test tissue responsiveness. Crude or purified PBTX was dissolved in methanol.

A concentration-response curve was determined for the crude PBTX and the purified fraction of PBTX having neurological activity.

The log concentration of PBTX needed to cause 50% of the maximal contractile response (EC_{50}) was determined for the crude and purified PBTX fractions.

Tachyphylaxis was measured by consecutive addition of PBTX to the tissue bath containing tissue, washing and allowing the tissue to equilibrate for 15-minutes between each exposure to the toxin.

All contractile responses were calculated in grams tension generated per gram tissue.

Testing Hemolytic Activity of PBTX on Canine Erythrocyte Solution—Hemolytic activity was tested with the procedure of Martin et al. (1972). Heparinized canine blood (5 cc) was washed twice with 20 mL of phosphate-saline buffer of the following composition (mM): $NaH_2PO_4 \cdot H_2O$, 4.0; Na_2HPO_4 , 210, in 0.9% NaCl solution, pH 7.5. Absorbances of canine erythrocyte suspensions were measured at 5-min intervals using an IBM model 9420 spectrophotometer. Test samples used 10 μ l of 10 μ g/mL methanolic toxin (crude or purified) per 3 ml of erythrocyte suspension. Control samples used 10 μ l of methanol.

Spectra—The UV-visible spectrum of 0.1 mg/ml of a purified PBTX fraction dissolved in methanol was determined using an IBM UV-visible spectrophotometer (model 9420). The spectrum was scanned from 185.0 to 450.0 nm.

Mass Spectrum—Crude PBTX was injected into the HPLC and fraction 2a was collected. The resulting fraction was then re-injected into the HPLC and recollected. The solution was evaporated and the residue, labeled twice-purified PBTX-2A-2x, was sent to Florida State University Mass Spectrometry Laboratory for molecular weight analysis. The sample was analyzed using positive chemical ionization mass spectrometry on a Finnigan model 4510 GC/MS spectrometer and scanning from 0 to 1050 E/M. Isobutane was the reactant gas and a solid probe (80-240/20) was used to introduce the sample into the system.

Statistical Methods—The Student's t-test was used for comparing unpaired data. The geometric mean was used to calculate the EC_{50} values from the concentration-response curves based on Fleming's (1972) method. Data are expressed as a mean plus or minus the standard deviation or standard error.

RESULTS—Purification of PBTX—The liquid chromatogram (Fig. 1) shows the three fractions eluted from crude PBTX. The first peak (fraction 1) appeared at 6-8% methanol and 94-92% chloroform. Fraction 1 was the largest peak on the elution profile and baseline separation was achieved. The second peak (fraction 2a) eluted at 15-16% methanol and 85-84% chloroform. The third peak (fraction 2b) eluted at 17-18% methanol and 83-82% chloroform. The retention time for each fraction was consistent from one run to the next. The retention times (71 sample injections) using a 20 μ l injection loop, for fraction 1, fraction 2a, and fraction 2b were (means $\pm$ S.D.) 2.6 ± 0.2 , 5.1 ± 0.2 , and 5.6 ± 0.2 , respectively.

Neurological Activity of PBTX—Data collected were used to construct a concentration-response curve for crude and purified PBTX fractions (Fig. 2). Fraction 2a had neurological activity (induced smooth muscle contraction); fraction 1 and 2b did not. An initial and final cumulative acetylcholine concentration-response curve (10^{-8} to 10^{-3} M Ch) was obtained for each tissue to test the consistency of the tissue responsiveness before and after exposure to PBTX. An EC_{50} value of 3.6×10^{-6} mol/L ($n=6$) was calculated from the initial cumulative acetylcholine concentration-response curve while the final EC_{50} value was 4.8×10^{-6} mol/L ($n=6$, $P=0.25-0.10$). The acetylcholine concentration-response curves before and after application of PBTX were unchanged in threshold, EC_{50} , or maximal contractile response.

The concentration-response curves for crude and purified PBTX were de-

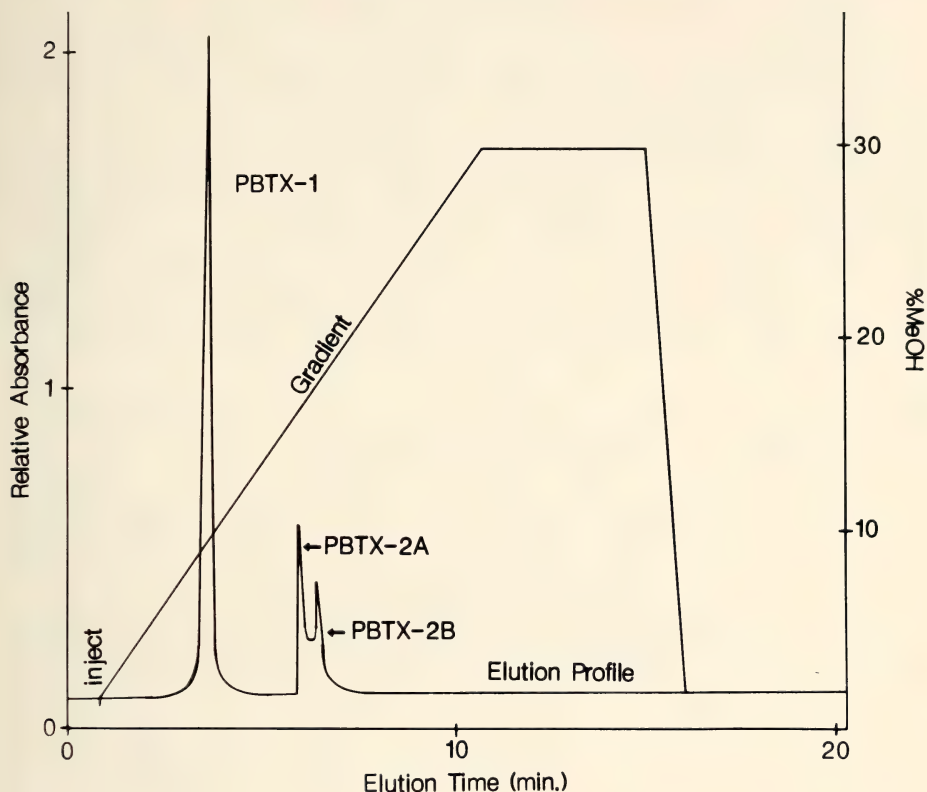


FIG. 1. Representative reverse-phase high performance liquid chromatogram of crude *Ptycho-discus brevis* toxin. The chromatograph was programmed to run a linear gradient for 10 minutes starting at 0% methanol and ending at 30% methanol in chloroform. Three primary peaks are seen on the elution profile. PBTX-1 had hemolytic activity, PBTX-2a had neurological activity, and PBTX-2b did not have hemolytic or neurologic activity.

terminated. The concentration-response curve for crude PBTX (Fig. 2) shows the maximum contractile response (mean $\pm$ S.E. = 170 ± 49 gram tension/gram tissue) occurred at a concentration of $5.0 \mu\text{g/ml}$. Following the first purification, a threshold contractile response occurred at a concentration of $0.1 \mu\text{g/ml}$ and a maximal contractile response (mean $\pm$ S.E. = 169 ± 56 gram tension/gram tissue) occurred with a concentration of $0.4 \mu\text{g/ml}$. The concentration-response curve of fraction 2a, after the second purification (Fig. 3) illustrates a threshold concentration of $0.01 \mu\text{g/ml}$ and the maximal contractile response (mean $\pm$ S.E. = 281 ± 40 gram tension/gram tissue) occurred at a PBTX concentration of $0.06 \mu\text{g/ml}$.

Analysis of the data obtained from contractile responses of canine tracheal smooth muscle strips showed an increase in the neurological activity resulting from purification of the toxin by HPLC. The EC_{50} values for successive purification were: $\text{EC}_{50} = 2.4 \mu\text{g/ml}$ (crude PBTX), $\text{EC}_{50} = 0.41 \mu\text{g/ml}$ (first purification), and $\text{EC}_{50} = 0.032 \mu\text{g/ml}$ (second purification). A six-fold

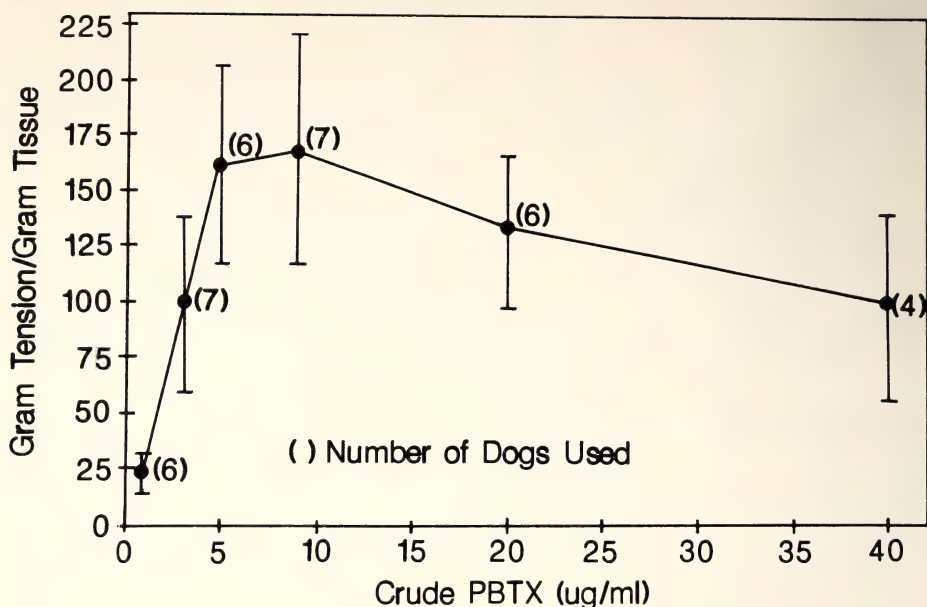


FIG. 2. Concentration-response curve of crude PBTX on canine tracheal smooth muscle strips. The threshold concentration of crude PBTX was $1.0 \mu\text{g/ml}$, and the maximum contractile response was induced by a concentration of $9.0 \mu\text{g/ml}$. Data illustrated are mean values plus or minus the standard error.

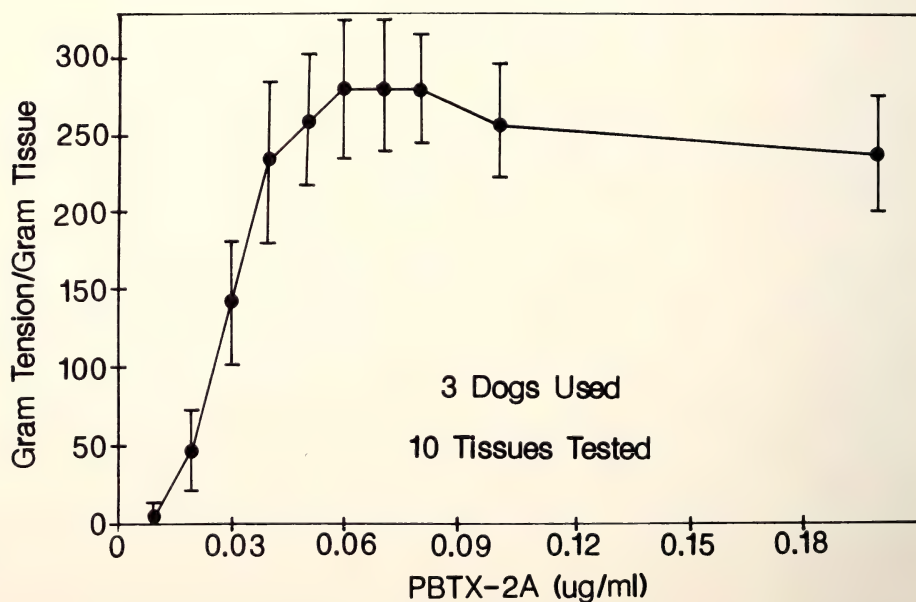


FIG. 3. Concentration-response curve of PBTX fraction 2a, after the second HPLC purification, on canine tracheal smooth muscle strips. The threshold concentration of fraction 2a was $0.01 \mu\text{g/ml}$, and maximum contractile response was induced by a concentration of $0.06 \mu\text{g/ml}$. Data illustrated are mean values plus or minus the standard error.

TABLE 1. Data analysis of neurological activity of crude and purified PBTX on canine tracheal smooth muscle strips.

Sample	Mean 50 % Maximum Response (g tension/g tissue)	EC ₅₀ (μg/ml)	Relative activity ^c to Crude PBTX
Crude PBTX	90 ± 20 ^a	2.4	1
PBTX-2a ^c	97 ± 29	0.41	6 ^b
PBTX-2a-2x ^d	157 ± 18	0.032	75

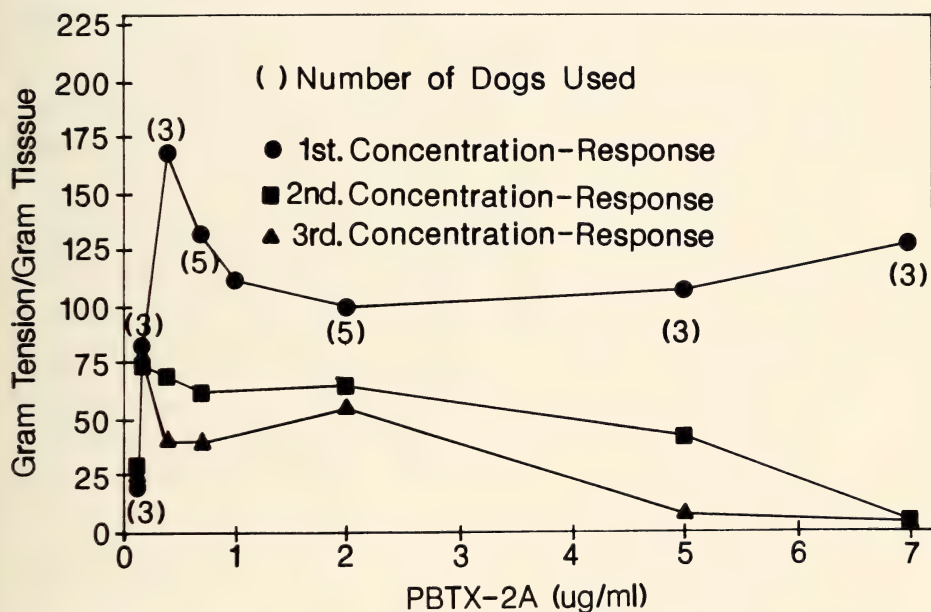
^amean plus or minus one standard error.^bratio of EC₅₀ values.^cPBTX Fraction 2a after the first HPLC purification.^dPBTX Fraction 2a after the second HPLC purification.^eThe neurological activity of Fraction 2a, and Fraction 2a-2x, relative to crude PBTX was calculated.

FIG. 4. Concentration-response curves of canine tracheal smooth muscle to crude PBTX fraction 2a, after the first HPLC purification. The smooth curve was generated 15 minutes after washout of and recovery from the first exposure to fraction 2a. The third curve was generated 15 minutes after washout of and recovery from the second exposure to fraction 2a. Data illustrated are mean values.

increase in neurological activity occurred with the first purification and a 75-fold increase in neurological activity occurred with the second purification (Table 1).

Consecutive Administration of PBTX to Canine Tracheal Smooth Muscle Strips—Canine tracheal smooth muscle strips were exposed three consecutive times to crude or purified PBTX to test for tachyphylaxis (rapidly developing tolerance). Figure 4 shows the phenomenon of tachyphylaxis which occurred with the addition of increasing concentrations of purified PBTX-2a. Administration of consecutive concentrations of fraction 2a showed that the average

contractile-response (g tension/g tissue) of the second (mean $\pm$ S.E. = 41 ± 33), and third (mean $\pm$ S.E. = 8 ± 6) concentration-responses were 38 and 7 % respectively, of the first concentration-response (mean $\pm$ S.E. = 108 ± 6), comparing at 5 μ g/ml of fraction 2a.

Hemolytic Activity of PBTX—The rate of hemolysis of canine erythrocytes was characterized as the pseudo-first-order rate constant, $k\psi$, which was taken as the slope of the linear relationship, $-\ln(A_t/A_{\infty})$ as a function of time (min.) Here, A_t and A_{∞} are, respectively, the absorbances at time t and at the end of the study period (typically several hours). The data were subjected to a least-squares treatment to obtain the slopes. The samples and values of $10^3 k\psi$, min^{-1} are: crude (8.5 ± 0.5), fraction 1 (16.1 ± 0.9), fraction 2a (2.8 ± 0.06), fraction 2b (2.9 ± 0.09) and control (2.6 ± 0.9). Analysis of these data show that only crude PBTX and hemolytic rate constants of fraction 1 were significantly different when compared with control values.

Analysis of Purified PBTX—The ultraviolet-visible spectrum of purified PBTX fractions showed a maximum absorbance of 208 nm for all three fractions. The spectrum did not have any other significant peaks in the visible region above 450 nm.

Also, the mass spectrum of fraction 2a, obtained after a second HPLC purification, was achieved with positive chemical ionization mass spectroscopy, using isobutane as the reactant gas and a solid probe to introduce the sample. The mass spectrum indicated that fraction 2a had a molecular weight of approximately 900 daltons, which is consistent with Brevetoxin B, reported previously by Lin and co-workers (1981).

DISCUSSION—Various HPLC solvent systems have been utilized to purify crude extracts of PBTX. Pierce and co-workers (1985) used isocratic elution with methanol and water. Also gradient elution systems have been utilized in which the solvent system becomes increasingly more polar. Success was achieved in separating the ichthyotoxic component from the hemolytic component of PBTX using methanol in chloroform (Padilla et al., 1979); a similar separation was described by Asai and co-workers (1982).

In the present study, a chloroform-methanol solvent gradient gave three fractions (Fig. 1). The chromatographic analysis indicated several useful observations. Fraction 1 was the most easily separated fraction in a given sample. Baseline separation was attained and was present in all crude PBTX samples injected into the HPLC.

In the present study, the neurological activity of HPLC purified PBTX increased. Following the first HPLC purification, the neurological activity of PBTX was increased six-fold, and a 75-fold increase in neurological activity of PBTX was observed after a second HPLC purification. Previous research with HPLC-purified PBTX on isolated canine tracheal smooth muscle (Asai et al., 1982), found a single neurologically active fraction and a hemolytically active fraction was not neurologically active. These results were confirmed in the present study: fraction 1 had hemolytic activity but no neurological activity.

PBTX caused canine tracheal smooth muscle contraction by stimulation of the sodium channels in the axons of the parasympathetic postganglionic nerves resulting in a release of the neurotransmitter acetylcholine at the neuroeffector junctions, thus contraction of smooth muscle (Asai et al., 1982). More specifically, PBTX appears to influence sodium influx at the h gate (Asai et al., 1984). Catterall and co-workers (1984) suggested that PBTX may act at a new receptor (receptor site 5) on the sodium channel. A similar sodium channel mechanism was proposed for rat vas deferens smooth muscle, an adrenergically mediated system (Sakamoto et al., 1985).

The consecutive contractile responses of canine tracheal smooth muscle strips to a constant concentration of crude PBTX or purified PBTX, show that tachyphylaxis occurred when relatively high concentrations of purified PBTX were utilized. A significant decrease in the contractile response, compared with crude PBTX, of consecutive second and third responses was observed using purified PBTX fraction 2a. Tachyphylaxis occurred when the concentration of fraction 2a was greater than 0.4 $\mu\text{g/ml}$. This tachyphylaxis could occur as a consequence of depletion of the transmitter acetylcholine; however, it is more likely that the red tide toxin which is highly lipid soluble is binding to tissue sites and inactivating them for a prolonged time period.

Other researchers have observed tachyphylaxis in both *in vitro* and *in vivo* systems using crude and purified PBTX. Asai and co-workers (1982) observed that at relatively high concentrations of crude PBTX (20 $\mu\text{g/ml}$), first-phase contraction of canine tracheal smooth muscle was followed by rapid return to baseline tension and the smooth muscle strips were insensitive to additional crude PBTX. Responses to acetylcholine were unaffected. Blockage of nerve firing was observed at high concentrations of purified PBTX using ventral nerve cord preparations of crayfish (Parmentier et al., 1978).

This study indicates that the contractile response of canine smooth muscle is the result of an action by a polyether with an approximate molecular weight of 900. The mass spectral results eliminate another structure that has been observed. Unfortunately, there are at least five polyethers with relatively similar structures: two are nearly identical, 11-fused polyether ring structures, differing only in a functional group at the terminal "K" ring on the fortieth carbon: Brevetoxin GB-2 is an aldehyde; and GB-3 is an alcohol (cf. Lin et al., 1981). In addition, Brevetoxin A, is said to have a similar ring structure but the terminal functional group is a $-\text{COCH}_2\text{Cl}$ moiety instead of an alcohol or an aldehyde. Two other functional groups are noted that are attached to the K-ring. The five structures, then, have a basically similar ring structure, that our experiments would not have distinguished. In addition, Brevetoxin A, said to be the most potent ichthyotoxin observed to date (Shimizu et al., 1986), has a 10-ring structure that differs from the other five polyethers both with respect to the number and size of the individual rings. Again, our results would not distinguish between this structure and the others.

Clearly, though we have separated a neurotoxic fraction, demonstrated

enhancement of activity, and identified an approximate structure, the actual compound or compounds responsible for the contractile response remain to be more adequately characterized. On the other hand, we may well wonder about the receptor site, and suggest that it may be more logical to presume the central role of an 11-ring structure in a receptor site than a single functional group at the K or J ring. Granted this hypothesis, it remains for future research to prove or disprove its validity.

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THE ANASTASIA ISLAND COTTON MOUSE (Rodentia: *Peromyscus gossypinus anastasae*) MAY BE EXTINCT—Stephen R. Humphrey,⁽¹⁾ William H. Kern, Jr.,⁽¹⁾ and Mark E. Ludlow,⁽²⁾ (1) Florida State Museum and Department of Zoology, University of Florida, Gainesville, FL 32611 and (2) Florida Department of Natural Resources, 13610 S.E. Federal Highway, Hobe Sound, FL, 33455

ABSTRACT: *The Anastasia Island cotton mouse appears to have been gone from Anastasia Island for several decades, but this status had not been acknowledged previously. A clear statement of the survival status of this subspecies is confounded by the problematic state of knowledge of its taxonomy. If its range is limited to Anastasia Island, the taxon is extinct. If the range includes Cumberland Island, Georgia, only the former population is extirpated. Though the mechanism of loss from Anastasia Island is unknown, much of the coastal forest habitat of this animal persists but is occupied by exotic black rats (*Rattus rattus*).*

THE cotton mouse on Anastasia Island, St. Johns Co., Florida, was named as *Peromyscus anastasae* by Bangs (1898) and assigned to *Peromyscus gossypinus anastasae* by Osgood (1909). Cotton mice on Anastasia Island occurred mostly in thickets of wax myrtle and Spanish bayonet and occasionally in the dune grassland (Bangs, 1898); based on habitat used by cotton mice elsewhere, maritime live oak forest probably also was occupied. Osgood also included in *P. g. anastasae* populations from Cumberland Island, Camden Co., Georgia, which had been named as *P. insulanus* by Bangs (1898).

The Anastasia Island cotton mouse is smaller and paler (more buffy in color) than typical mainland animals. The upper parts are brown on the mid-dorsal stripe and buff on cheeks, lower sides, inside of flanks, and base of tail, the underparts are grayish white, and the tail is dusky above and white below (Bangs, 1898). To this description, Osgood (1909) added that the combined populations of Anastasia and Cumberland Islands have upper parts "pale ochraceous buff rather lightly mixed with dusky, which is slightly or not at all concentrated in the mid-dorsal region."

The purpose of this study was to determine the current geographical distribution and survival status of the cotton mouse on Anastasia Island. The population was considered "status undetermined" by Neuhauser (1978). It is under review for possible listing as threatened or endangered (U.S. Fish and Wildlife Service, 1985).

METHODS—Sampling was conducted on Anastasia Island and vicinity from 22 May to 12 June 1986 (Fig. 1). Sites AI-9, 10, 13, 14, 15, and 17 were within Anastasia State Recreation Area. Sites AI-7, 8, 11, 12, and 16 and Site RI-1 were within Fort Matanzas National Monument. Sites CI-1, 3, and 4 and AI-3, 8, 9, 10, 11, 15, 16, and 17 were in wax myrtle, palmetto, xeric oak, or mesic oak habitats judged suitable for cotton mice. Cotton mice also are found occasionally in beach mouse habitat. Sites CI-2; AI-1, 4, 5, 6, 7, 12, 13, and 14; RI-1; and ML-1 were in dune grassland habitat judged suitable for beach mice. Site AI-2 was in dense grass judged suitable for cotton rats. Sampling also was conducted on the barrier beach from Vilano Beach northward from 12-20 June 1986. Sites SJ-3 to 9 were located in Guana River Wildlife Management Area. Sites SJ-4, 7, and 9 were in habitat suitable for cotton mice, and Sites SJ-3, 5, 6, and 8 were in habitat suitable for beach mice.

Trapping was done with lines of 40 large Sherman traps baited with rolled oats. To prevent mortality and saturation of traps by cotton rats, which are active at all times of day, traps were set in the late afternoon and checked and closed in the morning. Traplines were run for 1 to 3 nights. In reporting the sampling effort, trapnights were adjusted for sprung traps, counted as open for only half a night.

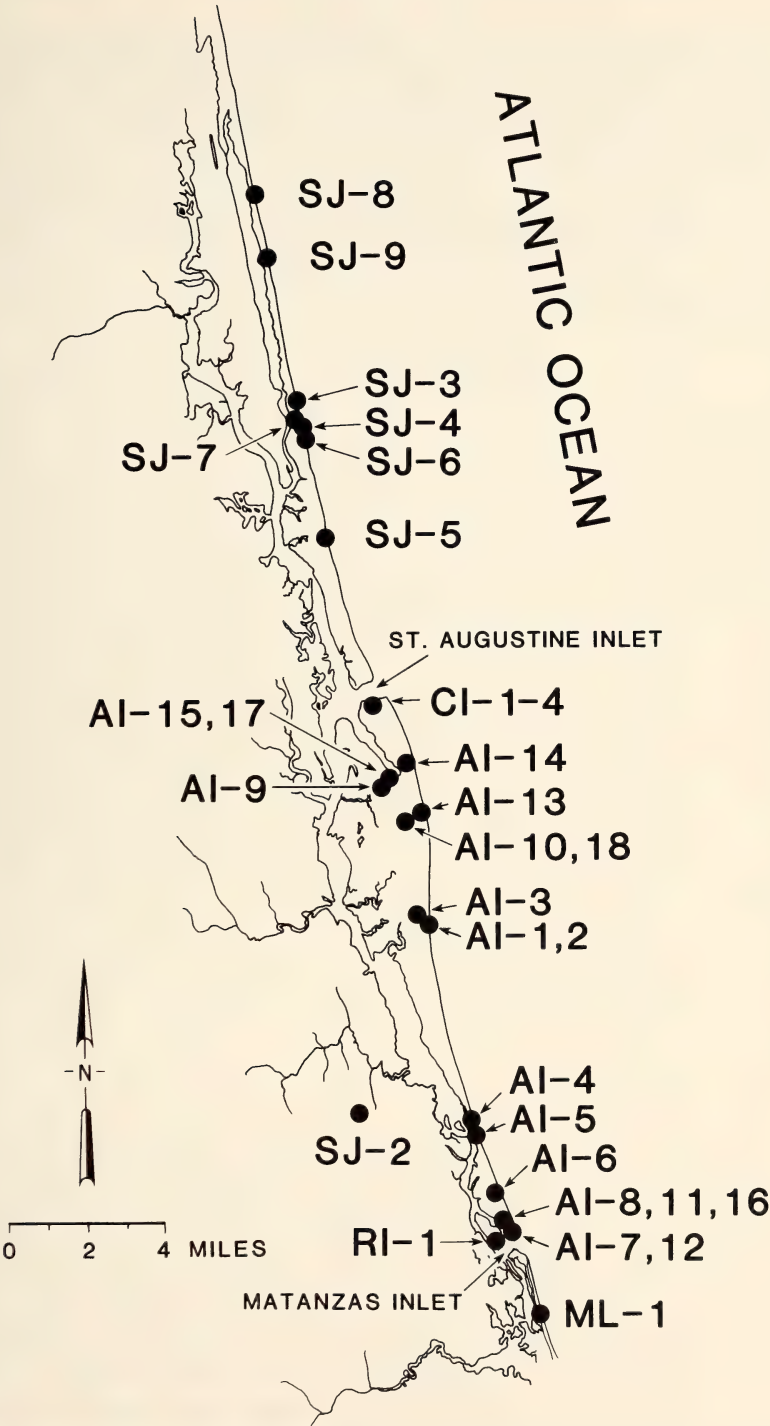


FIG. 1. Locations of sampling sites on Anastasia Island and northward in St. Johns Co., Florida.

RESULTS AND DISCUSSION—We captured no cotton mice on Anastasia Island in 880.5 trapnights in suitable habitat, or in over 2,000 trapnights of total sampling (Table 1). Our specimens of *P. gossypinus* from the barrier beach north of Vilano Beach (Table 2) were dark in color and not referable to the subspecies *anastasae*.

TABLE 1. Sampling of rodents with Sherman traps baited with rolled oats on Anastasia Island, nearby Conch and Rattlesnake Islands, and vicinity, 22 May to 12 June 1986.

Site	Adjusted trap nights	<i>Peromyscus</i> <i>gossypinus</i>	<i>Peromyscus</i> <i>polionotus</i>	<i>Sigmodon</i> <i>hispidus</i>	<i>Mus</i> <i>musculus</i>	<i>Rattus</i> <i>rattus</i>
CI-1	79	0	1	0	0	5
CI-2	76.5	0	8	0	2	0
CI-3	79	0	0	0	0	0
CI-4	80	0	0	0	0	0
AI-1	120	0	3	2	0	0
AI-2	120	0	0	13	0	0
AI-3	90	0	0	0	0	1
AI-4	20	0	0	0	0	0
AI-5	100	0	5	0	0	0
AI-6	121.5	0	2	1	0	0
AI-7	118	0	17	0	0	0
AI-8	108	0	0	10	0	1
AI-9	79	0	0	0	0	0
AI-10	73	0	0	0	0	0
AI-11	69.5	0	0	12	0	1
AI-12	79	0	7	3	0	0
AI-13	80	0	14	1	7	0
AI-14	69.5	0	7	0	3	0
AI-15	150	0	0	0	1	5
AI-16	34.5	0	0	9	0	1
AI-17	38.5	0	0	0	0	1
AI-18	38	0	0	0	0	0
RI-1	118	0	0	0	0	2
ML-1	39	0	0	2	0	0
SJ-1	60	0	0	1	0	0
SJ-2	38.5	0	0	0	0	0
Total	2,078.5	0	64	54	13	17

Cotton mice on Anastasia Island apparently were not common when first encountered—Bangs (1898) took only nine individuals. Elliot (1901, cited in Pournelle and Barrington, 1953) reported ten specimens taken at "Espanita", the home of a Mr. Middleton, located 2-3 miles (presumably north) from the type locality. Since 1901, at least four unsuccessful efforts have been made to find this population. Pournelle and Barrington (1953) failed to catch any in 306 trapnights in 1948, even though the purpose of their trip was to obtain topotypes. Smith (1987) failed to catch any in 3,553 trapnights in October 1978; this effort was directed at cotton mice and cotton rats, so trapping was in habitats other than dunes. Laerm (1987) failed to find this animal in approximately 5,000 trapnights on Anastasia Island in the early 1980s. Consid-

ering this history of trapping results, it is evident that the population on Anastasia Island has been extirpated for decades.

As the Anastasia Island taxon was originally conceived by Bangs, it was endemic to the island, and the present interpretation of its survival status would be that the taxon is extinct. However, the taxonomic revision by Osgood raised but did not resolve a taxonomic problem that makes the geographic status of this population (and hence its survival status) uncertain. If one accepts the current taxonomic arrangement established by Osgood (1909), *P. g. anastasae* is apparently extirpated from Anastasia Island but remains common elsewhere in its range (on Cumberland Island [Joshua Laerm, 1987]). If one rejects the current taxonomic arrangement, as we suggest, the Anastasia Island cotton mouse may be extinct.

Osgood referred only 18 specimens from Anastasia Island and 36 from Cumberland Island to *anastasae*. However, he examined *P. gossypinus* from other nearby areas, including St. Marys, Georgia; Amelia Island; Burnside Beach [on the coast of Duval Co. north of Anastasia Island]; Carterville [on the mainland west of Anastasia Island]; and Summer Haven [south of Matanzas Inlet]. Osgood's remarks about *anastasae* cast doubt on the validity of his taxonomic arrangement: "Although the pale forms from Anastasia and Cumberland islands, respectively, are entirely isolated from each other and from the mainland forms, they seem to be absolutely alike and also are not different from certain aberrant (intermediate?) specimens from the mainland. Moreover, the mainland specimens most similar to them are not from localities immediately adjacent to the islands in question, specimens from St. Marys, Ga., Burnside Beach, Fla., etc., being typical *gossypinus*." Further doubt about this arrangement can be inferred from Osgood's remarks under *P. g. palmarius*: "The type of *palmarius* and a very small percentage of the large series of topotypes are unusually pale and scarcely distinguishable from comparable specimens of *anastasae*. . . the great preponderance of dark specimens from the type locality [Oak Lodge, on East Peninsula at present-day Floridana Beach, across the intracoastal waterway from Micco, Brevard Co., Florida] tends to indicate that the type is probably an aberrant specimen rather than the representative of a well-defined form. The case might be construed also to the effect that pale coast forms are undergoing parallel differentiation at several points and that the same character (paleness) has been established independently on Anastasia and Cumberland islands and is only in its incipency on the peninsula opposite Micco."

This alternative interpretation notwithstanding, the taxonomic arrangement given by Osgood implies a range for *P. g. anastasae* extending far beyond Anastasia Island, including islands and unspecified portions of the mainland and excluding other portions of the mainland not known to be isolated by barriers to dispersal. This situation is unacceptably vague and contrary to common sense. We think the alternative suggested by Osgood—that such an arrangement may represent the lumping of relatively unrelated populations that are convergent in color, in adaptation to the light color of

TABLE 2. Sampling of rodents with Sherman traps baited with rolled oats on the barrier beach in St. Johns County from Vilano Beach northward from 12-20 June 1986.

Site	Adjusted trap nights	<i>Peromyscus</i> <i>gossypinus</i>	<i>Ochrotomys</i> <i>nuttalli</i>	<i>Sigmodon</i> <i>hispidus</i>	<i>Mus</i> <i>musculus</i>	<i>Rattus</i> <i>rattus</i>
SJ-3	40	0	0	0	1	0
SJ-4	34.5	0	0	6	0	0
SJ-5	119	0	0	5	1	0
SJ-6	116.5	0	0	9	1	0
SJ-7	115	0	0	5	0	3
SJ-8	78	1	0	9	1	0
SJ-9	72	7	1	2	0	3
Total	575	8	1	36	4	6

coastal soils—should be evaluated by a comprehensive study of geographic variation in *P. gossypinus*. Without good taxonomy, wildlife managers cannot determine what genetic entities are at risk.

Pournelle and Barrington (1953) cited development by man as the probable cause of the scarcity or absence of cotton mice at Anastasia Island. Though much of the habitat of cotton mice on Anastasia Island has been converted to human uses, substantial amounts of suitable habitat remain in small, fragmented patches. The identity of alternative threats is uncertain. One possibility is suggested by the fact that the remaining patches of suitable habitat now are occupied by black rats (*Rattus rattus*; Table 1). Though we have no direct evidence of competition between these two species, a causal relation between colonization of *Rattus* and extinction of *Peromyscus* is possible. For a parallel situation, refer to the case of the pallid beach mouse (Humphrey and Barbour, 1981). Research is needed on whether and under what conditions a strong competitive interaction of cotton mice and black rats is possible.

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A MODIFIED LIVE TRAP FOR THE CAPTURE OF SOUTHEASTERN POCKET GOPHERS—*Cyndi A. Gates¹, George W. Tanner, and Brian K. Gates*, Department of Wildlife and Range Sciences, University of Florida, Gainesville, FL 32611

ABSTRACT: A modified live trap for the capture of southeastern pocket gophers (*Geomys pinetis*) was developed for use in sandhill populations. Trapping success rate was 69%. The rate of plugging of traps by pocket gophers was lower and capture rate much higher than for Sherman pocket gopher traps.

ALTHOUGH a number of authors have reported on live-trapping of *Geomys bursarius* and *Thomomys* spp. in the western and central United States (Baker and Williams, 1972; Hart, 1973; Howard, 1952), little information has been published concerning live-trapping of the southeastern pocket gopher, *Geomys pinetis*. Southeastern pocket gophers were live-trapped between 2 February and 9 April 1986, in sandhill communities on the Katherine Ordway-Swisher Preserve in Putnam County, Florida. To accomplish this task, live traps were placed in the main tunnel systems of the pocket gophers. Main tunnels were located by clearing away soil of fresh mounds to expose the lateral tunnel (Brown and Hickman, 1973), and then following the lateral to the main tunnel. Care had to be taken to stay within the path of the lateral tunnel. Fragments of plant material gnawed off by the pocket gopher often were encountered and indicated that the excavator was following the right course. Average depth of main tunnel systems in this study was 29 cm, but ranged from 8 to 91 cm.

Two traps, placed as far into the burrow as possible and facing in opposite directions, were set at each site. A trap site was determined to be active if the presence of the animal could be detected; i.e. the trap had been sprung or the burrow or trap plugged with soil.

Initial trapping efforts which employed the use of Sherman pocket gopher traps, with the excavation site left open as suggested by Sherman (1941), yielded no captured animals. Of 12 total trap sets, 3 were active. In each of these 3 cases, the animal plugged the burrow and part of the trap.

Subsequent efforts involved setting Sherman-designed traps as above, but with boards placed over the top of the excavation and soil placed around the edges of the boards to insure that no light entered the burrow. Under these conditions, capture rate improved to 28% (2 of 7 active sets). However, the plugging of burrows and/or traps with soil was still a common occurrence. In 3 cases, animals completely filled the excavation, packing soil flush with the bottom of the boards.

Completely covering the trap, as noted by Howard (1952), and refilling the excavation site were important factors in increased trapping success. However, the treadle design of Sherman's trap does not lend itself to being covered with soil because of interference with the tripping mechanism. Therefore, a modified trap was designed to incorporate features that would

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allow complete coverage with soil (Fig. 1). The dimensions of the trap ($7.5 \text{ cm} \times 7.5 \text{ cm} \times 28 \text{ cm}$), the trap door ($7 \times 9 \text{ cm}$) and the hardware cloth floor at the front of the trap are features of the Sherman trap and were not altered. A pivoting paddle mechanism (A), similar in design to that used by Baker and Williams (1972), was attached to the top rear portion of the trap, 3.5 cm forward from the back wall. The paddle was suspended to within 4 or 5 mm of the trap floor. A stainless steel pin (B) held the paddle in place. A 3.2 mm diameter stainless steel wire rod (C), about 14 cm long, was inserted through the bottom corner of the paddle (D) and extended along the side wall to the top of the trap to a point about 11 cm from the front. The rod was held in place by a guide consisting of 3.2 mm diameter stainless steel wire (E) in such a fashion as to allow the rod to slide backwards when the bottom of the paddle mechanism was pushed back, thereby releasing the trap door (F).

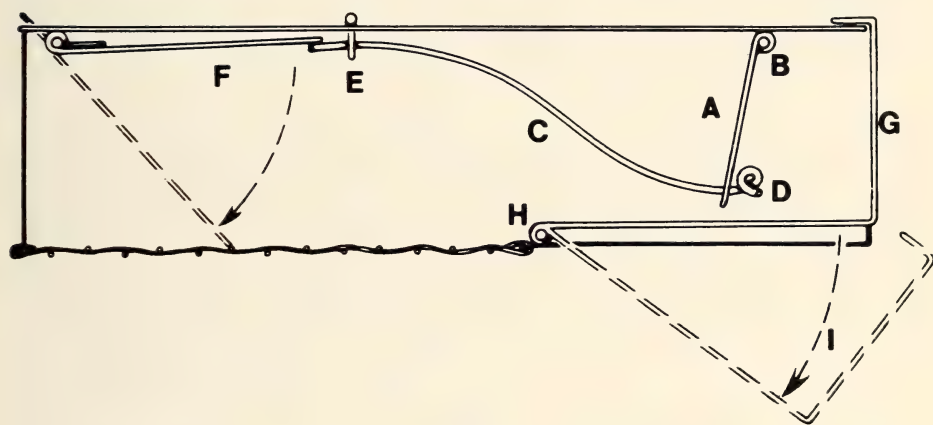


FIG. 1. Side view of the modified live trap for southeastern pocket gophers.

A single piece of galvanized sheet metal, with two right-angle bends, served as the floor and back wall of the trap (G). The floor of the trap was held in place with a stainless steel pin (H) so that it pivoted at its point of attachment to the trap walls. The floor and back wall component could be opened to facilitate removal of captured animals (I).

Forty-one pocket gophers were captured with the modified trap without mortality or injury. Trapping success was 69% (41 of 59 active sets), which was similar to that reported by Baker and Williams (1972) for *Geomys* spp. in Texas. The rate of plugging traps or burrows was much lower (30%) than with the standard Sherman trap. When all trap sets were counted (including those that were never revisited by the animal), the capture rate was 31%. Trapping success was greater when traps were left in place for 12-16 hours (66% of captures). There was a fairly low trap response rate overall (59 sets active of 130). Trapping response also declined with increasing dryness of the soil.

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CORRELATIONS AMONG EGG WEIGHT, CHICK WEIGHT, AND YOLK SAC WEIGHT IN BOBWHITE QUAIL (*COLINUS VIRGINIANUS*)¹

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ABSTRACT: *The relationships among egg weight, chick weight, yolk sac weight, and carcass weight (chick weight minus y.s. weight) in Bobwhite quail chicks at hatch were determined. Three egg weight groupings (9.6, 10.4 and 11.2 g) were used. Chicks were removed from the incubator at 4-hr intervals from the start of hatching and body and yolk sac weights were determined immediately. Larger eggs produced larger chicks with larger yolk sacs and larger carcasses. As egg size increased, the chick weight as a percentage of egg weight increased. Yolk sac as a percentage of chick weight and yolk sac as a percentage of carcass weight were significantly lower for chicks from small eggs. The following correlations were found: egg weight to chick weight, 0.88; egg weight to yolk sac weight, 0.38; chick weight to yolk sac weight, 0.37; egg weight to chick carcass weight, 0.73.*

EARLY mortality is a problem in commercially reared Bobwhite quail and appears to be more severe in the small quail chicks. In turkeys, early mortality from starve-outs has been shown to be higher with poults hatching from small eggs than large ones (Mayes and Auckland, 1976). Moran and Reinhart (1980) reported that the amount of residual yolk found in poults at placement was related to the weight of the egg prior to incubation. Not only were the yolk sacs heavier in the poults from larger eggs, but they also represented a larger proportion of the poult weight.

In addition to the important role the yolk sac plays in embryonic development, it is also presumed to be the primary nutrient source for chicks until placement (Romanoff, 1960). Harvey and co-workers (1955) deutectomized chicks and reported that they do not grow as well as normal ones for the first two weeks but were comparable thereafter.

Although the influence of egg size on the amount of yolk material available to chicks and poults at hatch has been well demonstrated, information on quail is limited. Studies on Japanese quail (Ricklefs et al., 1978; Roca et al., 1984) and on Bobwhite quail (Romanoff, 1944; Beane, 1981) suggest that as egg weight increases so does chick weight and yolk sac weight. It is not known, however, if the chicks from small quail eggs have proportionately less yolk available to them at hatch than quail from larger eggs.

If this relationship exists, it would help explain the high mortality observed in small quail chicks. This study was initiated to determine if the influence of egg size on the amount of yolk material available to the Bobwhite quail chick at hatch is consistent for all egg sizes. Additionally, the relationships among egg weight, chick weight, and yolk weight in Bobwhite quail were examined.

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METHODS—Eggs from 18-month-old Bobwhite quail breeders were collected daily and stored from 1 to 7 days at 12.8°C with a relative humidity of 80-85%. Prior to setting, all eggs were removed from the cooler, weighed to the nearest 0.1 g, and grouped into the following three weight categories: 9.6, 10.4, and 11.2g. A tolerance of plus or minus 0.1 g was allowed in each group. Eggs between these groups were discarded. The eggs were incubated at 37.8°C dry bulb and 30°C wet bulb temperatures. Incubation temperatures were changed to 37.2 and 33.3°C, respectively, on the twenty-first day.

After hatching was underway, chicks were removed from the incubator at four-hour intervals, killed by cervical dislocation, and weighed to the nearest 0.1 g. Yolk sacs were removed by tearing the skin from the abdomen and making an incision in the peritoneum along the midline, anterior to the umbilicus. The yolk sac was then forced from the body cavity by applying pressure to the sides of the abdomen. The yolk sac stalk was severed with forceps and the yolk sac (with contents) was removed and weighed to the nearest 0.01 g. Carcass weights were reported as chick weight minus the yolk sac weight.

Data was subjected to the general linear model and correlation procedures (Barr et al., 1976) with significant treatment differences determined by Duncan's multiple range test (Snedecor and Cochran, 1980).

TABLE 1. The influence of egg weight on chick weight, yolk sac weight and carcass weight of Bobwhite quail.

Egg weight (g)	(n)	Chick weight (g)	Yolk sac weight (g)	Carcass weight (g)	Chick weight as % of egg weight (%)	Yolk sac weight as % of chick weight (%)	Yolk sac weight as % of carcass weight (%)
9.6±0.1	43	6.8 ^a	0.79 ^a	6.0 ^a	71.1 ^a	11.5 ^a	13.2 ^a
10.4±0.1	96	7.4 ^b	0.95 ^b	6.4 ^b	70.8 ^a	12.9 ^b	15.1 ^b
11.2±0.1	100	8.1 ^c	1.08 ^c	7.0 ^c	72.2 ^b	13.3 ^b	15.6 ^b
Weighted average		7.6	0.98	6.6	71.4	12.9	15.0

^{a,b,c}Values within a column by different superscript letters are significantly different ($P \leq 0.05$).

RESULTS AND DISCUSSION—The average chick weight of 7.6 g (Table 1) corresponds exactly with the value reported by Beane (1981) for the same strain of quail and using a normal distribution of egg weight. Chick weight at hatch averaged 71.4% of the original egg weight. This is higher than the 66.2% value reported for Japanese quail (Ghany et al., 1966) and the normal range of 62 to 68% reported for chickens (Halbersleben and Mussehl, 1922; Morris et al., 1968; Upp, 1928). Romanoff (1944) reported the following values for Bobwhite quail: yolk sac weight of 0.49 g, yolk sac free carcass weight of 4.95 g, and the yolk sac represented 9.9% of the chick weight. These values are considerably lower than the corresponding values in this study .98 g, 6.6 g, 12.9%, respectively. This discrepancy may be due to differing strains of Bobwhite quail, differing incubation conditions, or the limited sample size of the earlier study.

Egg weight had a significant effect on chick weight, yolk sac weight, carcass weight, and chick weight as a percent of egg weight (Table 1). As egg weight increased so did chick weight, yolk sac weight, and carcass weight.

The larger egg weight produced a significantly higher chick weight as percentage of egg weight than the two smaller egg weights. A similar relationship between egg weight and chick and yolk sac weight has been reported for Japanese quail (Ghany et al., 1966; Ricklefs et al., 1978). Yolk sac as a percentage of chick weight or carcass weight (Table 1) was not significantly lower in chicks from the smallest egg group. This is similar to the results found with turkeys by Moran and Reinhart (1980).

TABLE 2. Correlation coefficients for egg, chick, yolk sac, and carcass weights.

	Egg weight	Chick weight	Yolk sac weight
Chick weight	0.88**		
Yolk sac weight	0.38**	0.37**	
Carcass weight	0.73**	0.86**	-0.16*

**Significant at ($P \leq 0.01$)

*Significant at ($P \leq 0.05$)

The correlation coefficient between egg weight and body weight was 0.88 (Table 2). Although this value is higher than the reported values of 0.72 and 0.77 for male and female Japanese quail, respectively (Ghany et al., 1966) and .55 for Bobwhite quail (Beane, 1981), a correlation coefficient of 0.91 was reported for Japanese quail by Ricklefs et al., 1978).

The correlation between egg weight and yolk sac was 0.38; the correlation between chick weight and yolk sac weight was 0.37. The similarity between these values resulted from the high correlation between egg weight and chick weight (.88). Parker (1929) reported that the correlation between egg weight and yolk sac weight was somewhat higher (0.48) in the chicken. Carcass weight was positively correlated with egg weight and chick weight, 0.73 and 0.86, respectively. A negative correlation existed, however, between carcass weight and yolk sac weight. Growth of the chick at the expense of the yolk sac may account for this effect.

The relationship among egg weight, chick weight, yolk sac weight appears to be similar to that reported for other avian species. More importantly, it appears that Bobwhite quail chicks hatched from small eggs have proportionately less yolk sac available to them at hatch than chicks hatched from larger eggs. Based on the importance of the yolk sac as stated by Romanoff (1960), this may partially explain the higher mortality observed in the smaller quail chicks.

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EFFECTS OF STOCKING DENSITY AND FOOD QUANTITY ON GROWTH OF YOUNG SNOOK, *CENTROPOMUS UNDECIMALIS*, IN AQUARIA

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ABSTRACT: *The purpose of this study was to determine the effects of food quantity and stocking density on the growth of juvenile snook. Two experiments were performed: 1) uniform stocking density (4 fish/38 L aquarium) and variable food quantity (7%, 10%, 20%, 30% body weight); 2) uniform food quantity and variable stocking density (1, 4, and 8, fish/38 L aquarium). Over ten weeks and 15 weeks respectively, standard length (SL) and weight were measured weekly. Growth rate was greatest in the 20% ration and groups of 4; least in 7% ration and groups of 8. Coefficient of Variation (CV) was greatest in the 10% ration and isolated fish; least in the 20% ration and groups of 4. Taken together, these measures suggest that the food quantity of 20% body weight and the rearing conditions in groups of four are most conducive to sustained maximal growth in aquaria.*

THE common snook, *Centropomus undecimalis*, is an extremely popular sport and food fish from the mangrove-lined estuaries of southwest Florida. This species is prevalent in the subtropical waters of South Florida around both east and west coasts.

The Florida Department of Natural Resources in 1985 implemented The Marine Fish Stock Enhancement Program out of concern for declining fish stocks (Edwards, 1985). A series of studies were initiated at the Mote Marine Laboratory (MML) to determine optimum rearing conditions for snook and to provide a basis for the establishment of an aquaculture program that could enhance recruitment to the snook fishery. The present investigation was designed to determine optimum stocking densities and food quantities for raising young snook to a size suitable for maximal survival upon addition to the snook fishery.

MATERIALS AND METHODS—Subjects were obtained from the Texas Palacios Hatchery Facility. Mote Marine Laboratory received the fish on October 15, 1985 when they were one month old. Experiments in the present study began when the fish were three months old, and had an average weight of 7.55 ± 1.70 g and a standard length of 78.7 ± 2 mm. Prior to experimentation the fish were maintained in a 177 L group tank and fed frozen brine shrimp twice daily.

Experiments were conducted on a closed-system aquarium table connected to a biofilter. The filter design employed a 192 L plastic container filled with perforated plastic spheres 5 cm in diameter to which was added Fritz-zyme^(TM), a saltwater conditioner. This provided bacterial substrates for processing of nitrogenous wastes. In all experiments the fish were housed in 38 L all-glass aquaria.

Fish were maintained at a salinity of 20‰ which was determined to be the optimal salinity for growth in another study at MML (Bruzek et al., 1987). The fish were kept on a 14/10 light/dark cycle under fluorescent lighting. Visual contact among fish in adjacent aquaria was prevented by opaque plastic film attached to the aquarium walls. Salinity, dissolved oxygen, pH and nitrates were measured daily.

Food in these experiments consisted of a ground mixture of equal portions of whole shrimp, fillet of mullet and tentacles of squid compacted into small cubes. Composition of this diet is shown

in Table 1. During the last week of experimentation a commercially prepared food (Ziegler's Salmon Moist) was administered to the fish to determine whether they would eat it. They consumed only negligible quantities after several attempts so further use of the commercial food was discontinued and the experiments were terminated. In this investigation we carried out two experiments. In one we sought to determine the optimum food quantity for individual growth at a constant stocking density. In the other we studied the effects of different stocking densities at the same food quantity per fish.

TABLE 1. Composition of diet fed to fish in Food-Quantity Study and Stocking-Density Study.

Ingredient (%)	Mullet	Squid Tentacles	Whole Shrimp
Carbohydrate	0	2.9	2.0
Fat	2.5	1.5	2.1
Protein	19.0	13.0	17.8
Ash	1.3	1.6	2.1
Calories/100 gram	104.0	80.0	103.0
Water	77.2	81.0	76.0

Food-Quantity Study—In this experiment, 32 fish were divided into groups of four and stocked in each of eight aquaria. The experimental treatment consisted of four different quantities of food. This provided two replicates of each treatment with a total of eight fish per condition. The amounts were adjusted as a percentage of average body weight per fish measured weekly (7%, 10%, 20% and 30%). Fish were fed twice daily, once in the morning and once in the afternoon. Feeding extended over a 15-minute period.

Stocking-Density Study—All fish were fed 20% of their average body weight twice daily on the same schedule as in the food ration study. Twenty-eight fish were assigned to 3 treatments in this experiment: 4 isolated fish, 2 groups of 4 and 2 groups of 8. The number of fish (N) in each condition was therefore 4, 8 and 16, respectively.

The dependent variables used to measure growth in both experiments were standard length (SL) and weight. A one-way completely randomized ANOVA for equal N was used in the Food Quantity Study and an ANOVA for unequal N was used for the Stocking Density Study to determine the significance of differences in mean length and weight. Tukey's Multiple Comparison Test (McClare and Dietrich, 1985) was used to determine which groups had significantly different measures of growth. Coefficient of variation (CV) of weight was also computed and changes in this measure of variability was noted over time in the groups. The CV of length showed the same results as CV of weight so we show only CV of weight. The means of initial weights and lengths of fish assigned to the treatments were compared in a one-way completely randomized ANOVA and there were no statistically significant differences.

Fish were weighed and measured once a week. They were anesthetized with MS-222 (tricaine methane sulfonate) at a concentration of 0.9 g/L, placed in a plastic mesh cylinder, then weighed and measured to the nearest 0.01g and 1.0 mm respectively.

RESULTS—Both length and weight were used as dependent measures of growth and the time courses of change were parallel. Weight tended to fluctuate more from week to week than length, and there were some drops in weight in both studies (Figs. 1A and 3A), Tukey's Multiple Comparisons Test on length showed the same results as the comparison on weight.

Food-Quantity Study—The mean weight and mean SL of the groups of fish fed 20% of their body weight increased the most. Fig. 1A shows that this treatment produced the greatest group increase of growth as well as the greatest absolute weight attained at the end of the study ($P < 0.01$). Their mean weekly growth rate was 0.70g and 1.9mm per week. The next greatest growth was attained by the 30% treatment, but it was not significantly dif-

ferent from the 20 % treatment ($P < 0.01$). The 10 % treatment showed significantly less ($P < 0.01$) growth than either the 20 % or 30 % treatments and the 7 % treatment showed the least (0.25g and 1.0mm). The 7 % and 10 % treatments showed no statistically significant differences from each other. The order of decreasing growth was as follows: 20 % > 30 % > 10 % > 7 %.

The coefficient of variation (CV) (Fig. 3A) shows a decline over time in the groups of fish fed 20 % and 30 % of their body weight. While the 30 % groups had a larger CV, these two treatments are not significantly different ($P < 0.01$) from each other. However, they have significantly smaller CV's than the fish fed 7 % and 10 % of their body weight ($P < 0.05$). The groups fed 10 % body weight increased the CV over time and ultimately showed the largest value, but it was not statistically significantly different from that of the 7 % groups which showed a similar increase over time. The treatments are arranged in the following decreasing order of CV: 10 % > 7 % > 30 % > 20 %.

A comparison of the CV with weight (Figs. 1A & 3A) shows that the groups fed the two greatest quantities had the lowest intra-group variability as well as the best growth. The 20 % group reached an asymptote of growth by the tenth week and showed no substantial change for the remaining weeks. The other three groups had a more gradual slope of growth continuing to increase until the 15th week. Also, the 7 % and 10 % groups fluctuated in weight throughout the study with weight losses as well as gains from week to week. Behavioral observations on the fish reveals some degree of agonistic behavior in all groups. But, since these behaviors were not quantified nor systematically observed, it is difficult to assess the hypothesis that the 7 % and 10 % groups spent more time fighting than eating and thus periodically lost weight. Also, there was no mortality in this study so the potential effect of the absence of measurements of large fish could be ruled out. Figs. 1A & 3A shows that coefficient of variation of the treatments could be segregated into two sets of two treatments with high values of one variable and low values of the other. That is, both 20 % and 30 % treatments showed a low CV and high growth while the 7 % and 10 % treatments showed a high CV and low growth.

Stocking-Density Study—Examination of both measures of growth, weight and SL (Figs. 2A & B) show essentially the same results. Tukey's test on both weight and SL indicated that Groups of Four grow best (0.55g and 1.3mm/wk, $p < 0.05$) followed by Isolates. Groups of Eight grew the least (0.16g and 0.6mm/wk, $p < 0.05$) but their growth was not statistically significantly different from the Isolates.

The coefficient of variation (CV) was significantly larger in Isolates than in the other groups ($P < 0.01$). Groups of Eight had a larger, but statistically indistinguishable, CV from that of Groups of Four which had the lowest CV. The decreasing order of CV is: Isolates > Groups of Eight > Groups of Four. Comparison of CV and weight (Figs. 2A & 3B) showed a roughly inverse relationship similar to that which appeared in the Food-Quantity Study. In the Stocking-Density Study, Isolates increased CV over time to the highest level and have intermediate growth. The Groups of Four showed the best

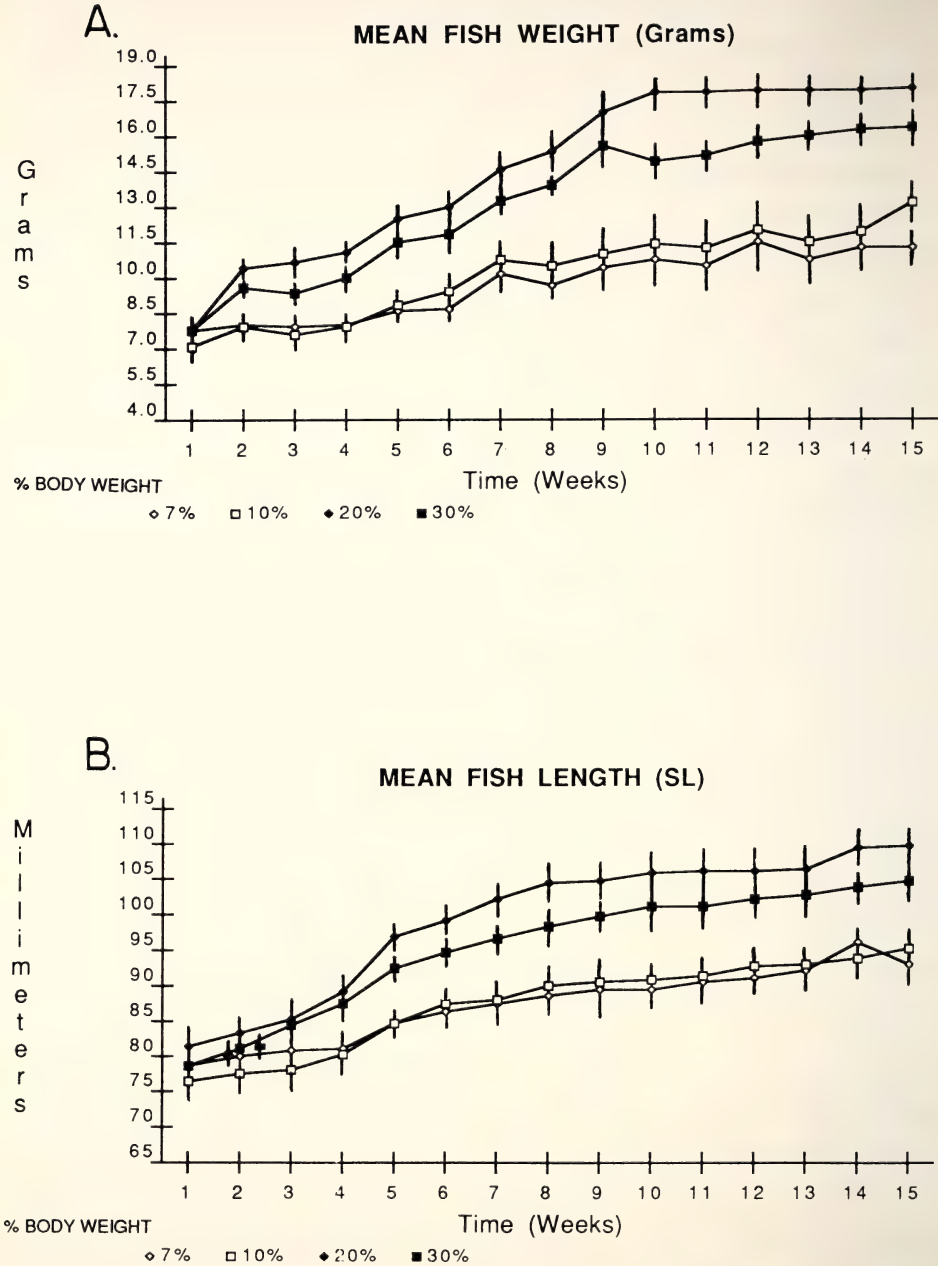


FIG. 1. (A) Mean weight of fish (grams) measured weekly in Food Quantity Study. (B) Mean standard length (SL) of fish (mm) measured weekly in Food Quantity Study. Treatment groups were fed 7%, 10%, 20% and 30% of their mean body weight twice daily and stocked in groups of four fish per aquarium.

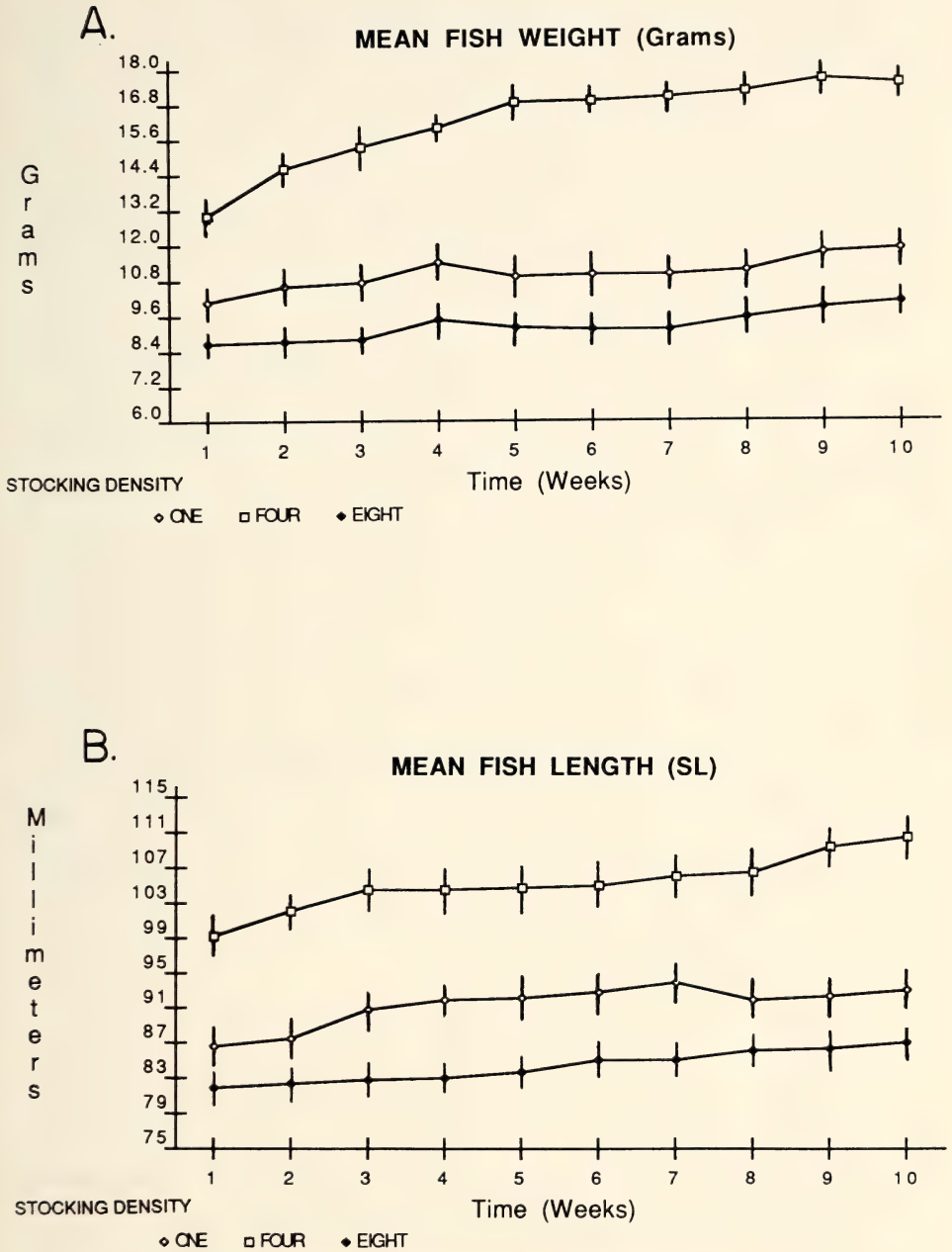


FIG. 2. (A) Mean weight of fish (grams) measured weekly in Stocking Density Study. (B) Mean standard length (SL) of fish (mm) measured weekly in Stocking Density Study. Treatment groups were 4 Isolates, 2 Groups of Four and 2 Groups of Eight fish per aquarium all fed 20% of their mean body weight twice daily.

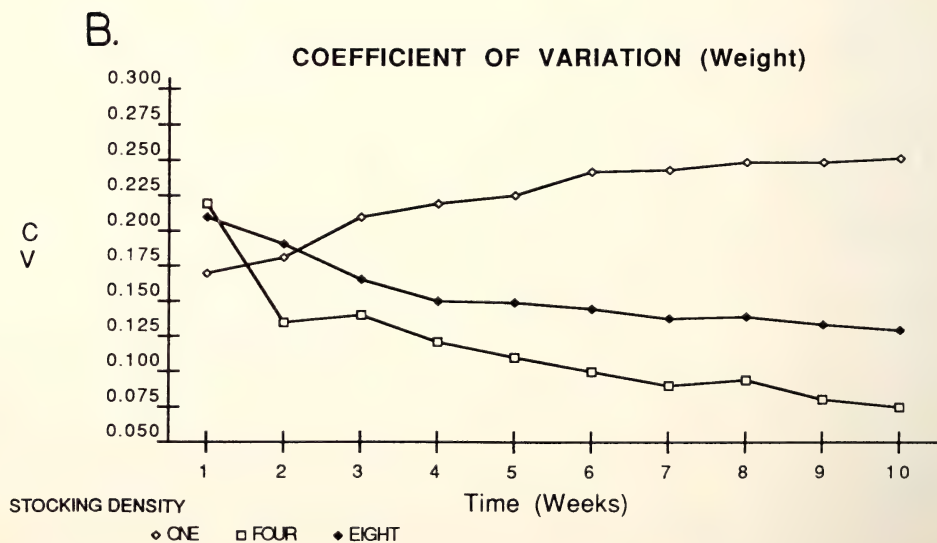
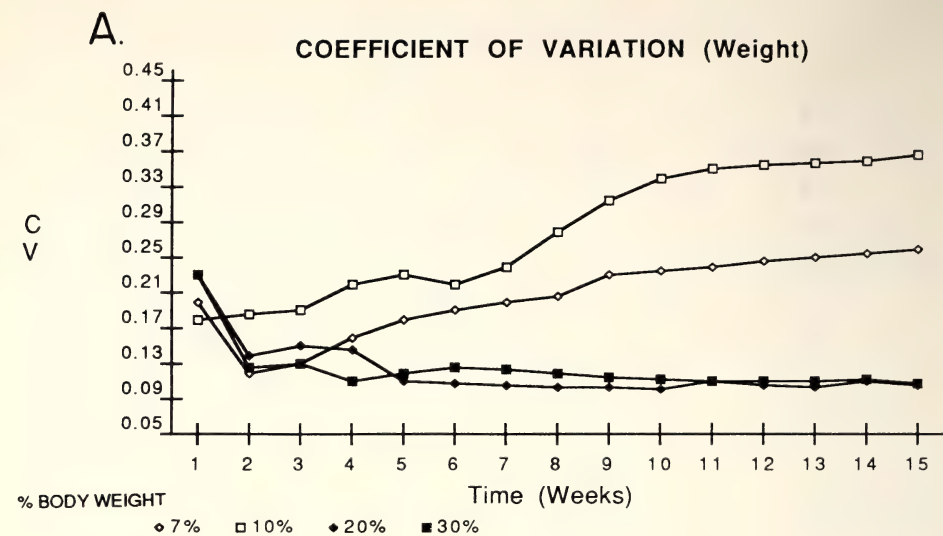


FIG. 3. (A) Coefficient of variation of weekly weight measurements of fish in groups fed 7%, 10%, 20% and 30% of mean body weight in Food Quantity Study. (B) Coefficient of variation of weekly weight measurements of fish maintained as Isolates, Groups of Four and Groups of Eight in Stocking Density Study.

growth, and they showed a decline in CV over time culminating in the lowest value. Groups of Eight showed the least growth among the three treatments, and they had a declining CV which was of intermediate value, but statistically similar to that of the Groups of Four. Also, the only mortalities observed in these experiments occurred in the Groups of Eight in which two fish died.

DISCUSSION—We found the clearly optimal level in the factors that we studied. For example, the mean weight and SL was greatest in the fish fed 20 % of their body weight daily. Groups receiving this treatment also exhibited the lowest coefficient of variation. The optimal social condition for growth apparently was Groups of Four. They grew better than Isolates or groups of Eight. The best combination of food quantity and diet was 20 % body weight and Groups of Four.

The fluctuations in growth exhibited by the groups of fish fed 7 % and 10 % of their body weight parallel their high intra-group variation measured each week as shown by the CV. Since the stocking density was the same for all groups (four per group), differential social environment can be ruled out as a factor. No quantitative behavioral measurements were made, so the presence or absence of dominance hierarchies cannot be determined. The relative position of the mean weekly growth curves for the four treatments would imply that food quantity was limiting below the 20 % ration but, on the other hand, that an excess beyond this was not associated with greater growth. Further, this excess ration produced a decrease in intra-group variation. This suggests that the agonistic behavior observed in all groups may reflect some type of "scramble" competition which was more intense in the groups fed the two smaller food quantities than in the 30 % group. It is not clear why this latter group should have less growth than the 20 % group since the presence of food in excess would effectively reduce or eliminate competition for food and each fish should have access to the food quantity that would maximize growth. There was no difference in water quality due to the possible effects of unconsumed food in the 30 % group because all groups were on the same recirculating water supply.

Groups of Four in the Stocking-Density Study showed the greatest growth as well as the least intra-group variation. The greatest rate of increase in growth took place during the first five weeks of the study and then increased more gradually until the end of the study. Growth curves of the other two treatments do not show any particular period of maximum rate of increase but rather a gradual overall increase over the course of the study interspersed with periods of growth stasis or actual decline. The greater decline of intra-group variability in the Groups of Four vs. Groups of Eight suggests that in these two treatments where social interaction is possible, the average fish in the Groups of Four was getting more to eat than one in the Groups of Eight but not more than any of its tank mates. Again, a correlation of social organization such as dominance hierarchies with access to food and hence growth cannot be reliably established in the absence of behavioral data.

A comparison of growth rates of fish in the present experiments with those of pond-reared snook juveniles reveals a substantially lower growth rate in aquarium-raised fish. Bruzek and co-workers (1987) obtained maximum rates of 2.03g/week and 4.6mm/week in fish fed a "natural" diet compared with our best growth rate (20% treatment) of 0.70g and 1.9mm/week. They measured and weighed fish every 30 days for 180 days; we handled them every week. It is possible that handling may be stressful and interfere with growth. Of course, the confinement in aquaria may not facilitate maximum growth.

Many factors may interact to affect growth. These include temperature and food quantity (Brett, 1969); light (Pyle, 1969); and oxygen (Allen, 1974). In this investigation, all these variables were held constant. However, social interaction has been implicated in a few previous studies on growth of fishes (Brown, 1946). An initial assumption in such studies is that at any particular density or food ration all the fish can eat an equal quantity of food. This should lead to uniform growth among fish exposed to the same conditions. When this result is not obtained, that is, great variation in individual growth within a treatment is observed, many investigators assume that social interaction has intervened. These investigators postulate that social structure may directly or indirectly affect equal access to food or in some other way affect growth. They term this the "size hierarchy effect" (Brown, 1946; Nagoshi, 1967; Koebele, 1985) and assume that a dominance hierarchy has formed such that large individuals can dominate a social situation. Even when food supply is not limiting, such individuals may grow larger and negatively affect the growth of subordinates. Further, they formulated a measure termed "growth depensation" which represents a temporal increase in variance of a size frequency distribution due to individual differences in growth rates.

In the present investigation, we used coefficient of variation (CV) as a measure of variability within groups and in our analysis made no assumptions concerning dominance hierarchies. Because individual fish were not marked and no systematic behavioral observations were carried out other than to note the presence or absence of agonistic behavior, we believe that assumptions concerning social organization are premature. If limitation of access to food due to interference competition were a factor and/or a dominance hierarchy formed, one would expect stocking densities of eight fish to show the largest CV, but in fact this did not occur. Under the conditions of our experiments, it does not seem that individual variation in growth can be reliably associated with social conditions. We suggest further work in which the existence of a dominance hierarchy by behavioral tests would be established independently and correlations with variation in growth could then be determined.

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ARCHAEOLOGICAL TESTING AT THE ANDERS SITE: A WEEDEN ISLAND-RELATED MIDDEN ON BOCA CIEGA BAY, ST. PETERSBURG, FLORIDA

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ABSTRACT: *Limited test excavations were conducted at a small shell midden located near the shore of Boca Ciega Bay in St. Petersburg, Florida. Ceramics recovered from the site indicate that the midden was deposited during the late Weeden Island-related period, or about A.D. 800-1000. A pre-midden, pre-ceramic occupation is also evident which may date as early as the middle to late Archaic period, or about 4000-2000 B.C. Analysis of the subsistence remains indicates a heavy reliance on shellfish, particularly oyster (*Crassostrea virginica*) and bay scallop (*Aequipecten irradians*), with only minor evidence for other marine or terrestrial species.*

THE Anders site (8Pi1252) was brought to the attention of the author by its owner, Mr. Dan Anders, who found shell and bone while digging in his yard to install a well and sprinkler system. The bone material was identified by the author as fragments of the skull and femur of a sub-adult human between the ages of 10 and 15 years old. Mr. Anders described the stratigraphic context as consisting of approximately one foot (0.3 m) of topsoil and sand overlying about 0.3 m of compact shell. The skeletal material was found near the bottom of the shell deposit.

A visit to the site indicated that while located in a residential neighborhood, enough of the site remained intact to warrant the excavation of a small test unit to determine whether or not a prehistoric cemetery was present, and to gather culture-historical and subsistence data from the midden.

The Anders site is located in what was once a dense oak/palm hammock along the eastern shore of Boca Ciega Bay near the mouth of Long Bayou (Fig. 1). Modern dredge and fill activities have altered the shoreline somewhat, but the original topography can still be discerned. The site originally sat on a low dune ridge which sloped gradually to the west toward the bay. What may have been a small lagoon is still visible to the west-southwest despite modern filling.

PREVIOUS ARCHAEOLOGICAL INVESTIGATIONS—The eastern shore of Boca Ciega Bay was densely populated by prehistoric aborigines, especially during the late Weeden Island-related and Safety Harbor periods (ca. A.D. 800-1700). The accounts of early pioneers and archaeologists alike describe the many large shell mounds and middens that once lined the shore for several kilometers. The area may have been visited by Europeans as early as 1528 when the Spanish explorer Pánfilo de Narváez landed on the Pinellas peninsula. Some scholars feel that Narváez landed somewhere on the eastern shore of Boca Ciega Bay (e.g. Swanton, 1946:37), perhaps at a large temple mound and village located about 1.8 km south of the Anders site.

Despite the area's rich history and prehistory, there have been few controlled archaeological investigations at these sites. Discounting the early digging done by explorers, adventurers and the locally curious, the earliest reported investigations of these prehistoric communities are those by Walker (1880a, 1880b), Moore (1900) and Bushnell (1926). Of these, C. B. Moore was the most prolific digger. Unfortunately, his field methods were primitive by today's standards, and his reports are of questionable utility beyond providing maps and a brief description of some of the more spectacular artifacts.

In modern times only two reported excavations have been conducted in the central bay area near the Anders site. William Sears (1960) excavated a burial mound, portions of a large temple mound and a nearby shell midden at the Bayshore Homes site located just 0.37 km north of the Anders site. Based on these excavations, Sears felt that the site dated to the latter part of the Weeden Island (related) period. The site was significant in that it contained a temple mound, normally associated with later, Safety Harbor period sites, in an apparent Weeden Island-related context.

The Narváez site, so named because of its possible association with that explorer, was test excavated by Frank Bushnell who reported his findings in 1966. This large shell mound, which is probably a temple mound with associated plaza and midden, is securely dated to the Safety Harbor period. Historic period artifacts found by Bushnell and other collectors, including sherds of Spanish Olive Jar, iron nails, and fragments of an iron chisel, constitute the material evidence for inferring this as Narváez' landing site.

Surface collections and uncontrolled digging by artifact collectors have occurred at many other sites along this part of the bay. All of the artifacts tend to confirm the Weeden Island-Safety Harbor temporal association for most of these sites. Today nearly all of the area is residential.

METHODS—A one by one meter square test unit was excavated as near as possible to the location where Anders found the skeletal material, situated so as to avoid obvious recent disturbance from the well, septic tank and pipelines (Fig. 1). Natural and/or cultural strata were used as the primary recovery units during excavation. Within each stratum, excavation proceeded by arbitrary 10 cm levels to maintain vertical control. All excavated soil was screened through 0.64 cm hardware cloth.

All artifacts and bone material were collected and bagged by level. Notes were made on the shell species observed and their relative abundance in each level. Soil profile maps were drawn of the south and west walls of the unit, and photographs were taken of the stratigraphy. Three column samples consisting of approximately 500 cc of soil each were collected from the midden zone. These were later wet sieved through fine-mesh window screen in an effort to obtain very small faunal material or seeds.

Stratigraphy—The soil profile shown in Fig. 2 is relatively simple and straightforward. Zones 1 and 2 represent the upper humic zone and the underlying disturbed soil zone, respectively. Zone 3, the midden deposit, consisted of abundant shell mixed with a dark grayish brown, organic sand matrix. Beneath the midden deposit was a light tan to white fine sand (Zone 4).

The upper disturbed zone is the result of ground clearing and fill deposits associated with the construction of the Anders' house. A few pieces of aboriginal ceramics were recovered from the bottom of this heavily mottled soil zone. From an examination of the profile, it appears that the upper portion of the midden was disturbed slightly during construction, which resulted in the mixing of prehistoric ceramics with modern fill.

The midden deposit lacked internal stratification; however, a heavy concentration of scallop shells was encountered in the north half of the unit at a depth of about 48 cm. No charcoal was

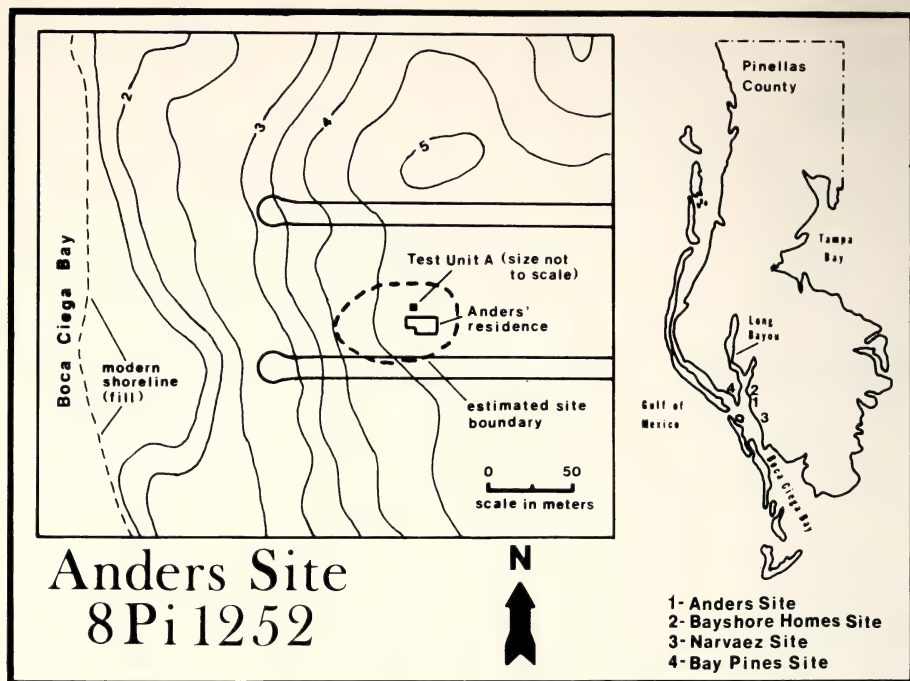


FIG. 1. The Anders site showing the location of Test Unit A, the Anders' residence, and topographic features. Accompanying map of Pinellas County shows the location of sites mentioned in text.

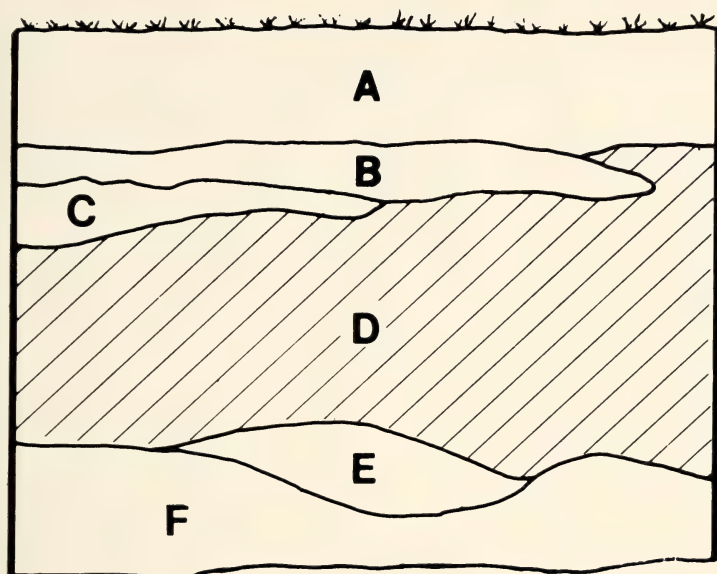
intermixed with the shell, nor were the shells blackened from roasting. The concentration probably represents a single discard episode of shells from scallops opened and consumed at a nearby location. Ceramics and shell tool fragments were found sparsely distributed throughout the rest of the midden.

The midden deposit rests on what was once the original ground surface. Except for two small ceramic sherds at the very top of this sand zone, it contained only lithic debitage. There is the suggestion of a shallow pit feature underlying the midden (Fig. 2), but no artifacts or faunal material were associated with this feature.

Artifacts—Prehistoric ceramics recovered from the site include St. Johns Plain, limestone-tempered plain, two varieties of sand-tempered plain, a laminated, contorted paste ware, and a possible Carrabelle Punctated sherd. Also recovered were several sherds that appear to be variants of the Ruskin Linear Punctated type described by Willey (1949:442). The sherds display a series of short, linear rows of small, triangular to semi-circular punctations created using a jab-and-drag technique. Often rows of punctations intersect one another. The paste of these sherds is very sandy and their surfaces appear to have been smoothed before any decoration was applied.

The laminated, contorted paste sherds appear with the naked eye to be temperless; however, on microscopic examination they are found to contain a small amount of fine quartz sand and small clay lumps as inclusions. Another, possibly related, plain ware was also recovered. This is a more obviously sand-tempered ceramic, with a hard, compact paste containing occasional clay lumps, and a well smoothed surface. Two of these sherds display fine lamellae in cross section, but are dissimilar in all other respects to the heavily contorted and laminated sherds described previously.

Table 1 presents a list of the ceramics recovered and their proveniences. Most were recovered from the midden zone. A few small sherds of unglazed, red earthenware in Zones 1 and 2 represent the only material evidence for historic occupation. Prehistoric sherds in Zone 2 are the result of post-depositional disturbance. The two sherds at the top of Zone 4 are probably intru-



- A** - Zone 1 topsoil & sandy humus
B - Zone 2A mottled gray & tan sand
C - Zone 2B mottled lt. gray & white sand
D - Zone 3 midden - dk. gray-brown sand
 & shell
E - Zone 4A mottled brown, tan & gray sand
F - Zone 4B white, tan & orange-brown sand

0 10 20
 Scale in cm.

FIG. 2. West wall profile of Test Unit A. Note shallow depression underlying the midden zone (Zone 3).

sions from the midden above, but they could represent the initial ceramic period occupation of the site which resulted eventually in the creation of the shell midden.

Non-ceramic artifacts from the midden consist almost exclusively of implements manufactured from shell including a *Busycon* celt fragment, a shaped *Busycon* whorl fragment that may have been used as a ladle, several *Busycon* columellae that appear to have been utilized as hammers or punches, and a few miscellaneous pieces of ground shell (*Busycon* and *Mercenaria*).

Also recovered from the midden was a chert core fragment. The only non-ceramic artifacts recovered from the underlying sand zone consist of lithic debitage (primarily small thinning and retouch flakes) and a single bifacial tool fragment.

TABLE 1. Vertical distribution of ceramics, Test Unit A

Ceramic Types	Stratigraphic Zones								Totals
	1	2	3			4			
	Excavated Levels								
	1	2	3	4	5	6	7	8	
Historic Terra Cotta	2	3	1						6
St. Johns Plain		3		2	1		1		7
Carabelle Punctated		1							1
Ruskin Linear Punctated				1	3	2			6
Sand-tempered plain ^a				1	3		1		5
Sand-tempered plain ^b					12	3			15
Laminated, contorted ware				1	1	1			3
Limestone-tempered					1				1
Totals	2	7	1	5	21	6	2	0	44

^alarge amount of quartz sand in paste

^bhard, compact paste with sand and clay lumps

FAUNAL ASSEMBLAGE—The faunal assemblage is dominated by the presence of shellfish remains, principally oyster (*Crassostrea virginica*) and bay scallop (*Aequipecten irradians*). During excavation it appeared that scallop shells were the dominant species, however this was probably due to the shell feature described above. Species data from the column samples indicate that oyster actually accounted for the greatest percentage of the total shell weight, while scallops accounted for the highest MNI total (Table 2). Together, these species account for over 75% of the total shell weight recovered from the column samples.

The amount of non-shell faunal material is surprisingly low. Given its bayside location, an abundance of bone material reflecting exploitation of a marine environment was expected. In fact, only 7.3 gm of bone were recovered from the column samples. This corroborates observations made during excavation; that is, that bone was noticeably scarce in the midden.

Despite the relatively small sample, a number of different species were identified representing a variety of habitats (Tables 2 and 3). Soft-shelled turtle (*Trionyx ferox*) and cooters/sliders (*Pseudemys* sp.) both inhabit fresh-water ponds and streams. Gopher tortoise (*Gopherus polyphemus*) and white-tail deer (*Odocoileus virginianus*) inhabit the interior hammocks with the gopher tortoise often found in open, sandy areas. All of the fish species identified are common estuarine species, as are stone crab and all of the shellfish. No deep water fish, such as redfish or black drum, were present in the sample suggesting that marine fishing was confined to nearby estuaries.

TABLE 2. Species list for column samples, Test Unit A

Species	MNI	Wt. (in gm)	Cum. Wt. (in gm)	%	Cum. %
Invertebrates					
Oyster (<i>Crassostrea virginica</i>)	9	94.3	94.3	51.64	51.64
Bay Scallop (<i>Aequipecten irradians</i>)	11	39.7	134.0	21.74	73.38
Lightning Whelk (<i>Busycon contrarium</i>)	5	8.5	142.5	4.65	78.04
Banded Tulip (<i>Fasciolaria hunteria</i>)	3	7.0	149.5	3.83	81.87
Quahog Clam (<i>Mercenaria</i> sp.)	2	15.8	165.3	8.65	90.53
Crown Conch (<i>Melongena corona</i>)	1	0.2	165.5	0.11	90.64
Fighting Conch (<i>Strombus alatus</i>)	1	0.4	165.9	0.22	90.85
Common Slipper (<i>Crepidula fornicata</i>)	7	0.3	166.2	0.16	91.02
Slipper Shell (<i>Crepidula</i> sp.)	11	0.7	166.9	0.38	91.40
Beaded Periwinkle (<i>Tectarius muricatus</i>)	1	0.4	167.3	0.22	91.62
Unidentified	—	9.2	176.5	5.04	96.66
Vertebrates					
Hardhead Catfish (<i>Ariopsis felis</i>)	1	0.1	176.6	0.05	96.71
Sea Catfish (Ariidae)	3	0.2	176.8	0.11	96.82
Sheepshead (<i>Archosargus probatocephalus</i>)	1	0.1	176.9	0.05	96.88
Mullet (<i>Mugil</i> sp.)	2	0.3	177.2	0.16	97.04
Unidentified Bony Fish (Osteichthyes)	—	3.7	180.9	2.03	99.07
Unidentified Shark (Chondrichthyes)	1	0.2	181.1	0.11	99.18
Unidentified Turtle (Testudines)	4	0.3	181.4	0.16	99.34
Unidentified Vertebrates	—	1.2	182.6	0.66	100.00
Totals	63	182.6	182.6	100.00	100.00

TABLE 3. Vertebrate species list for excavated levels, Test Unit A

Species	Fragment Count	MNI
Hardhead Catfish (<i>Ariopsis felis</i>)	6	2
Mullet (<i>Mugil</i> sp.)	2	2
Unidentified Bony Fish (Osteichthyes)	12	1
Stone Crab (<i>Menippe mercenaria</i>)	4	3
Gopher Tortoise (<i>Gopherus polyphemus</i>)	1	1
Cooters/Sliders (<i>Pseudemys</i> sp.)	4	2
Soft Shell Turtle (<i>Trionyx ferox</i>)	1	1
Unidentified Turtle (Testudines)	6	3
White Tail Deer (<i>Odocoileus virginianus</i>)	3	2
Unidentified Mammal	7	3
Unidentified Vertebrate	6	—
Totals	52	20

The presence of mullet (*Mugil* sp.) and catfish (*Ariopsis felis* and Ariidae family) are suggestive of mass capture techniques using nets. The absence of juveniles and small-sized fish species in the fine-screen column samples further suggests that something akin to a gill net was used as opposed to a fine-mesh net.

DISCUSSION—Based on the ceramic assemblage, occupation of the Anders site occurred principally during the late Weeden Island-related period, or approximately A.D. 800-1000. Carrabelle Punctated ceramics are good temporal markers, having been found in Weeden Island contexts at many sites along the Gulf Coast and the interior of north-central Florida (Willey, 1949:425; Milanich et al., 1984:64-65). The jab-and-drag ceramics, if they are indeed a variant of Ruskin Linear Punctated, should also date to this cultural period (Willey, 1949:442). The contorted paste sherds may be a variety of Pinellas Plain, a later, Safety Harbor period ware that is often sand- or grit-tempered, and is identified by its laminated, contorted paste (Willey, 1949:482; Luer and Almy, 1980:209). The sherds reported here, however, are softer than typical examples of Pinellas Plain.

It is not unusual to find Pinellas Plain ceramics in Weeden Island-related contexts in the Tampa Bay area. Sears (1960) reports finding hard, laminated sherds of Pinellas Plain in the lower levels of the Bayshore Homes midden in association with such Weeden Island-related types as Ruskin Dentate Stamped. In the upper levels of the midden, laminated sherds are soft and many "may even be temperless" (Sears, 1960:4). These last sherds sound similar to the laminated, contorted ware from the Anders site. Thus, the association of Pinellas Plain-like, laminated and contorted paste sherds with Carrabelle Punctate and Ruskin Linear Punctate ceramics supports a late Weeden Island-related date for the Anders midden. In all likelihood, the site was contemporaneous with the upper levels of the Bayshore Homes midden.

An earlier, pre-ceramic occupation (pre-2000 B.C.) is suggested by the presence of lithic waste flakes, and an absence of ceramics and dense midden deposits in the lower levels of the excavation. Unfortunately, no temporally diagnostic artifacts were recovered from these lower deposits, so this temporal assignment remains unconfirmed.

The faunal assemblage suggests that the site's inhabitants exploited a number of different environments for food: estuarine, freshwater, and interior ridges and hammocks. All of these environmental zones can be found within a short distance of the Anders site. All of the vertebrate and molluscan species could have been taken year round, although deer were traditionally hunted during the fall and winter months when meat weight was highest and the deer populations less dispersed (Hudson, 1976:275; Larson, 1980:52).

The most complete presentation of quantitative subsistence data (both vertebrate and invertebrate) from the lower Pinellas Peninsula that is available in published form is from a late Archaic (ca. 2100 B.C.) midden at the Bay Pines site (Braley, 1978), located 2.4 km west of the Anders site on Boca

Ciega Bay. Oyster and scallop were the dominant shellfish species there, and these were supplemented with fish, turtle and deer. This assemblage is similar to that from the Anders site which suggests that relatively little change in environment and subsistence strategies occurred during the nearly 3000 year period that separated the two occupations.

Oyster and scallop appeared to be the primary food staples at Bay Pines; however, using standardized food value tables (Watt and Merrill, 1963), Braley (1978:48-52) calculated the dietary values of the site's constituent species to determine their relative importance to the aboriginal diet. His analysis showed that deer contributed the bulk of the caloric intake, with fish providing the highest amount of protein. These same tables were used to calculate dietary values for the faunal species collected from the Test Unit A column samples at the Anders site (Table 4). Although the sample is small and not

TABLE 4. Dietary values of fauna from column samples, Test Unit A

Fauna	MNI	Avg. live _wt. (kg)	% Usbl. wt.	Cal./ 100 gm	Total cal.	Gm. pro./ 100 gm	Total protein
Oyster	9	0.01	1.00	66	59.40	8.4	7.56
Scallops	11	0.01	1.00	112	123.20	23.2	25.52
Turtles	4	1.59	0.50	100	3180.00	19.2	610.56
Fish	7	1.32	0.55	103	5234.46	17.6	894.43
Clam	2	0.3	1.00	80	480.00	11.1	66.60
Whelk	5	0.9	1.00	98	4410.00	18.7	841.50

completely representative of aboriginal subsistence at the site (for example, no deer remains were identified in the column samples), the results do demonstrate the nutritional importance of non-shellfish species, particularly fish and turtle. Although shellfish played an important role in aboriginal subsistence by providing easily obtainable food in bulk, their nutritional importance was relatively minor when compared to other species, particularly deer. To illustrate, the remains of at least two deer were recovered from the general excavation levels of Test Unit A. Their estimated caloric value equals about 80,000 calories. In order to obtain the same number of calories from shellfish, it would be necessary to consume over 7100 scallops or 12,000 oysters.

No human skeletal remains were recovered during the excavation. The shallow depression observed in the west wall profile could be the remains of a burial pit associated with the child's skeleton found by Anders. If so, the interment must have occurred prior to the deposit of the midden. This does not seem likely since Anders reported finding the skeletal material in a shell matrix; however, shell from the overlying midden could have become mixed with burial fill when Anders excavated his trench.

Although it is possible that a cemetery is (or was) located nearby, isolated burials or individual skeletal elements are not unusual in shell middens. At

the Bay Pines site human skeletal elements were often found intermixed with midden shell (Gallagher and Warren, 1975:106, 110; Braley, 1978:44). That these may represent "parts for whole" burials (a term coined by Lyman Warren) is a speculation that has some basis in fact. Single skull interments, for example, are not uncommon in late prehistoric burial mounds (cf. Willey, 1949; Bullen, 1952; Sears, 1967). However, a "parts for whole" explanation implies a high degree of mortuary ritualism that is belied by the fact that the disarticulated elements occur in strikingly non-sacred contexts, that is, in kitchen middens. A more likely explanation is that under certain circumstances human remains were disposed of in an unceremonious fashion. For instance, continuous reoccupation of a site over several centuries could have resulted in the disturbance of earlier burials and these would need to be discarded. Periodic cleaning of a charnel house would also result in the collection of disarticulated elements in need of disposal.

Complete isolated burials in midden contexts have been reported at the Sellner Midden in Hillsborough County (Bullen, 1952:72) and the Askew Site in Citrus County (Bullen and Askew, 1965:204-205), to mention only two. At the latter site the burial was that of a sub-adult. Again, the fact that the interments occurred in otherwise secular contexts suggests that the individuals were not of high status and that elaborate mortuary ceremonialism was not practiced. They may represent the burials of individuals who resided at these sites, or they may have been persons who were accorded low status in their society, or were considered "unclean" because of illness or infirmities, and were thus denied access to more conventional resting places such as burial mounds or cemeteries.

CONCLUSIONS—Although limited in scope, archaeological testing at the Anders site resulted in useful chronological and subsistence information for an area that has seen little in the way of controlled excavation. Analysis of the excavated artifacts indicates that the site was contemporaneous with the nearby Bayshore Homes site, a large town center occupied during the late Weeden Island-related period. The individuals who resided at the Anders site were probably part of the surrounding Bayshore Homes village complex.

ACKNOWLEDGMENTS—Species identification of the vertebrate faunal sample was conducted by Karen Jo Walker at the Zooarchaeology Range of the Florida State Museum, Gainesville. Her comments in correspondence to the author were used extensively in the descriptive discussion of the faunal assemblage. Food value calculations were conducted by the author and he is solely responsible for any misapplication or misinterpretation of these data. The author would also like to extend his appreciation to Dana Ste. Claire for assisting in the field work, and especially to the Anders family for allowing us to disrupt their backyard for one Saturday in the interest of learning more about St. Petersburg's past.

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RANGE EXTENSION OF *IPOMOEA TURBINATA* LAG. (CONVOLVULACEAE) TO SOUTHERN FLORIDA—⁽¹⁾Daniel F. Austin and ⁽²⁾Richard K. Jansson, ⁽¹⁾Department of Biological Sciences, Florida Atlantic University, Boca Raton, Fl. 33431, and ⁽²⁾University of Florida, I.F.A.S., Tropical Research & Education Center, 18905 S.W. 280 St., Homestead, FL 33031.

ABSTRACT: A range extension is reported (from the Panhandle to southern Florida) for the purple moonflower (*Ipomoea turbinata*). The species, originally perhaps from Mexico, has been established elsewhere in the southeastern U.S.A. for some years.

SPREAD of the purple moonflower (*Ipomoea turbinata* Lag.) in North America has largely gone unnoticed until recently. As with many species either cultivated or spread as contaminants in crop seeds, there are often few records of range extension and/or establishment. Often the arrival of a species in any particular area goes unnoticed until it becomes established. Gunn (1970) was apparently the first to record the establishment of the species in the United States as a weed. Being spread as a contaminant in soybean seeds, the species became established in the lower Mississippi valley and spread to the Carolinas by the early 1970's.

Although Gunn (1972) was of the opinion that the species had been naturalized in Mexico, its biological affinities indicate that it was most likely native to that country (Austin, 1982). Indeed, the species was probably taken from the Americas and naturalized in Africa and India. In Florida, the plants are easily recognized in flower by their purple salverform nocturnal corollas with caudate external sepals. Fruiting specimens may be separated from *Ipomoea alba* by their strongly reflexed and thickened pedicels; pedicels are neither reflexed nor thickened in *I. alba*.

Few records are available for the species in southern Florida. Plants have been cultivated here for some years, but these have largely been ephemeral. Since the species is more adapted for temperate conditions, it was not expected to become established in southern Florida. The only collections examined from the wild until recently were those from the Florida panhandle (Leon Co.) in the herbarium at Tallahassee (cf. Clewell, 1985: 335).

While examining, collecting, and identifying various *Ipomoea* species for studies (by RKJ) concerned with host range, habitat, and distribution of the sweetpotato weevil, *Cylas formicarius elegantulus* (Summers) (Coleoptera: Curculionidae) in southern Florida, purple moonflower was found and identified (by DFA). The vine was found growing wild along the edges and within the canopy of lime trees in southern Dade Co. (Homestead). Most plants showed signs of weevil damage [a large (40 ha.) boniato (*Ipomoea batatas*) field was located adjacent to the lime grove within 25 m. of the purple moonflower patches]. Fifteen plants were removed and cultured in plastic pots in a glasshouse for further study.

This note is the first record of the arrival and range extension of the purple moonflower in southern Florida.

VOUCHER SPECIMEN: Florida. Dade Co. Growing as a weed in a lime grove, SW 192 St. between 187 & 177 Ave, Homestead, Florida. June 1987. *D. F. Austin, R. K. Jansson & S. H. Lecrone 7518* (FAU).

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REVIEW

Jonathan J. Becker. *Neogene Avian Localities of North America*. Smithsonian Institution Press, Washington D. C., 1987. Pp. 171. Price \$14.95.

Neogene Avian Localities of North America is an annotated synopsis of all published North American Miocene and Pliocene localities that have produced bird fossils. Most localities are from the United States, but a few are from southern Canada and northern Mexico. Dr. Becker's purpose for writing the book is to correct the widely scattered and sometimes inaccurate reports in the literature of Neogene birds, and to compile them faunistically. The book's main selections include (1) a check-list of North American Neogene birds, (2) North American Neogene avian localities (Late Arikareean, Hemingfordian, Barstovian, Clarendonian, Hemphillian, and Blancan), (3) an extensive literature cited section, and (4) charts and maps showing chronology of faunas and distributions of localities that have produced fossil birds. The locality section makes up most of the book's text. Each entry includes the name of the fauna, the geological formation of the political state, the relative age based on evolution of land mammals or marine correlative, dates, a list of the avian taxa, and references. Some of the taxa used by Dr. Becker are different from those of *The A. O. U. Check-List of North American Birds* (6th Ed.). For example, Accipitriformes is used instead of Falconiformes, Pandionidae instead of Pandioninae, Ralliformes instead of Gruiformes, and Parulidae instead of Parulinae. I found the book interesting, attractively assembled, and easy to survey. I am sure students of avian paleontology will find the synopsis worthwhile and will want to purchase it at the modest price of \$14.95.—Walter Kingsley Taylor, University of Central Florida, Orlando.

FUNGAL FILAMENTS IN
MILLEPORA COMPLANATA Lamarck, 1816
(Cnidaria: Hydrozoa)
AFTER MASS EXPULSION OF ZOOXANTHELLAE

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ABSTRACT: *Fungal filaments from Millepora complanata Lamarck were observed after mass expulsion of zooxanthellae. The hyphae were characterized using light and scanning microscopy and Gomori's methenamine silver stain.*

ALTHOUGH microbial diseases are relatively common in Scleractinian reef corals from environmentally stressed areas (Peters, 1984; Antonius, 1985; Glynn et al., 1985), there is scant evidence of disease-related eukaryotic microbial filaments in coral tissue. Ramos-Flores (1983) suggested that a lower marine fungus with non-septate hyphae was closely associated with "black line" disease in the star coral *Montastraea annularis* Ellis and Solander. Recently, a disease called "black band" disease in Atlantic reef corals was identified as *Phormidium corallyticum* Rutzler and Santavy (Cyanobacterium, Oscillatoriaceae) (Rutzler and Santavy, 1983; Taylor, 1983; Rutzler et al., 1983). Although macroscopic symptoms may appear similar, associated microbes can vary widely (Peters, 1984).

Paleoecological examinations of a variety of marine sediments revealed from their casts an array of microborings. Fungal hyphae were reported to be among them (Golubic et al., 1975; Budd and Perkins, 1980). Fungi have been reported from a variety of other carbonate substrates including crustacean and foraminiferian tests as well as mollusk shells (Kohlmeyer and Kohlmeyer, 1979; Kohlmeyer, 1985; Porter, 1986). Kendrick and co-workers (1982) isolated saprobic fungi from the interior of the aragonite skeleton of living corals in the Caribbean and the South Pacific. The propounded role of fungi in bioerosion in marine environments seems indisputable.

A major zooxanthellae expulsion event occurred in coral reefs seaward of Key West, Florida during early September, 1983 (Jaap, 1985). Unusually warm calm weather and low tides coinciding with midday combined to elevate seawater temperature, thus stressing the reef corals. Perhaps ultraviolet radiation, reduced dissolved oxygen, and increased salinity also induced stress in these reef communities. This event provided an opportunity to evaluate the occurrence of fungal filaments which were associated with the diseased reef corals, especially *Millepora complanata*.

MATERIALS AND METHODS—Habitat and substrate characteristics: Greatest incidence of coral bleaching was in the shallow spur and groove habitats at Eastern, Middle, and Western Sambo Reefs, Eastern Dry Rocks, Rock Key, and Sand Key Reefs. The most intense discoloration occurred in <3 m depths; however, bleached corals were observed to 12 m depths in the forereef habitat. *Millepora complanata* was the dominant cnidarian in the shallow reef habitat; at Eastern Sambo Reef it comprised 32.5% of the community (N = 20 one m² quadrants); there were 11 other species and 209 individual colonies at the time of the event. The *M. complanata* manifested approximately 75% incidence of discoloration; they appeared bone-white in contrast to their normal golden-yellow color. A post-event survey at seven months revealed about 10% mortality of the *M. complanata* population. Specimen preparation: Bleached and normal colored specimens of *M. complanata*, *Acropora palmata* Lamark and *Palythoa caribbaeorum* Duchassaing and Michelloti, as well as specimens of *Madracis mirabilis* Duchassaing and Michelloti, a species that appeared to retain its normal color, were collected from Eastern Sambo Reef (24° 20.6'N, 81° 39.8'W) off Boca Chita Key, Monroe County, Florida on 6 October 1983. Specimens were fixed in Zenker's formalin for 12 h, washed for 24 h, and stored in 70% non-denatured ethanol (Yevich and Barszcz, 1977). Calcium carbonate skeletons and spicules were removed with sodium citrate-formic acid. Tissues were washed in tap water for 12 h and stored in 70% ethanol. Processing for histological evaluation was done in a histomatic tissue processor on the following schedule: 95% ethanol—2 changes, 1.5 h each; 100% ethanol—3 changes, 1 h each; xylene—2 changes, 1 h each. Paraffin infiltration was done under vacuum with two changes, 1.5 h each. These blocks were sectioned at 6 μ m and sections mounted on slides from a water bath containing 0.0125% gelatin. The slides were subsequently dried overnight in a 55 C oven over formalin fumes. Sections were stained by Movat's modified pentachrome stain (Yevich and Barszcz, 1977) for general structure and Gomori's methenamine silver stain for fungi (Sheehan and Hrapchak, 1980). Specimens were examined for pathological features with light microscopy. Subsequently, a specimen of *M. complanata* was dehydrated into 100% ethanol, frozen in 100% ethanol with liquid nitrogen, fractured, critical point dried, mounted on an SEM stub, and gold coated. This material was examined and photographed with a Hitachi HHS-2R scanning electron microscope (SEM).

RESULTS—Normal-colored specimens of the species examined, except *M. mirabilis*, were void of microbial filament assemblages. It appeared that these specimens retained their zooxanthellae. Those filamentous forms seen in *M. mirabilis* tissue samples appeared to be algae. The filaments were smooth, did not evidence any branching and were light green using light microscopy. No microbial filaments were observed in bleached *A. palmata* or *P. caribbaeorum* tissues, but bleached specimens of *M. complanata* contained massive populations of filaments that appeared to be fungal hyphae (Fig. 1), based on the response to Gomori's methenamine silver stain as well as morphological features. Intercellular filaments with differing diameters occurred; one ranged from 0.9 to 2.8 μ m and the other ranged from 4.0 to 6.6 μ m. Branching was seen in both the larger and the smaller filaments. Septations, although present (Fig. 2), were not seen in all segments of the filaments.

DISCUSSION—Based on these limited observations the filaments observed in the bleached tissues of *M. complanata* are probably saprobic fungi. The state of experimental pathogen studies of corals is in its infancy. Kendrick and co-workers (1982) proposed an extant population of fungi in several corals from the Barbados and South Pacific including *M. complanata*. This was based on obtaining a number of fungi in culture from the interior of the argonite skeletons of these corals. They gathered specimens from natural communities and then returned to the laboratory to obtain inocula from the

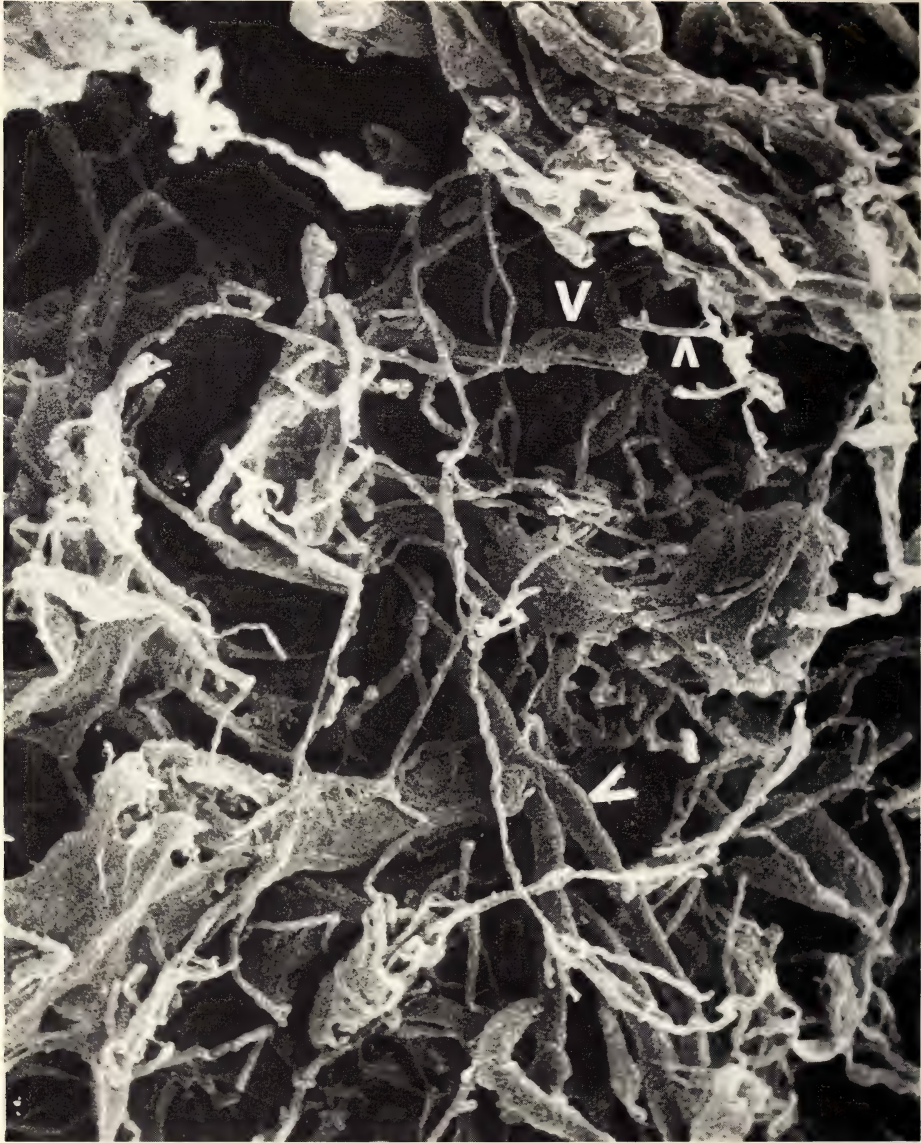


FIG. 1. Scanning electron micrograph of *Millepora complanata* tissue with a complex of small (small v's) and large (large v's) diameter microbial filaments thought to be fungi. (X1000)



FIG. 2. Light micrograph of *Millepora complanata* tissue and hyphal filaments with septa (arrows). Material was treated with Gomori's methenamine silver stain used for fungi. (X450)

interior of each skeleton sample. These inocula showed that several fungi would grow from samples derived from *Siderastrea siderea* Ellis and Solander. Our studies did not indicate that hyphae could be readily seen in normal-colored *M. complanata* or the other specimens examined. These differences are puzzling and certainly support the need for continued investigations.

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